

Rothschild Deserves a Few Friends

THE Select Committee on Science and Technology has missed the point of Lord Rothschild's proposals for the reorganization of that part of British publicly supported research. In the first of three documents on the organization of research (see pages 63 and 64), the committee accepts the more obvious criticisms of the tactless green paper which has kept the laboratories buzzing since November, which has provoked indignant protests from the research councils, made august advisory committees stand on their dignity and helped to swell the correspondence columns not merely in *Nature* but the daily newspapers as well.

It is right to say, as the committee does, that the Rothschild report appears too much like a rabbit from a hat, that its incorrigible author should have consulted those affected more thoroughly in advance (if only so as to have disarmed the criticisms which have since been made of him), that he should have produced more evidence to support his chief proposal that a large part of the research carried out by the research councils should be conceived of by government departments and paid for by means of negotiated contracts with the councils, that some of his arithmetic was incorrect, that his report should have covered the whole of civil research and that the government has only confused the issue by endorsing the "customer-contractor principle" at a time when it could have had only the vaguest and most doctrinaire idea of what that meant. The committee might have added that the report was written in needlessly inflammatory language. But that does not excuse its failure seriously to address the principal questions raised by Rothschild—first the extent to which the deliberate commissioning of research by government departments might help to make some British research more pointed and second the nature of public accountability in basic research. If the green paper was indiscreet, the committee's commentary is vapid.

The committee's chief counter-proposal is in its way as much of a rabbit from a hat as any of the Rothschild recipe. The committee accepts the complaint that under present arrangements there is insufficient connexion between the needs of government departments and the work sponsored by the research councils. It adds, quite properly, that the machinery for deciding where money should be spent should cover not merely the work of the research councils but of the other publicly supported civil research, that of the Atomic Energy Establishments and the industrial laboratories now under the Department of Trade and Industry. Even defence research should be accommodated. But then the committee stumbles on to say that there should be a Minister for Research and Development, with a seat in the cabinet, with his own budget and with authority to scrutinize and approve the plans for research and development of all government departments. To give advice on priorities, there would be a Council for Science and Technology, and it is only fair to say that the committee is right if it implies that

there is a need of an independent talking-shop much broader in its coverage than the present Council for Scientific Policy. But what makes the committee think that a minister, even a cabinet minister, will be more sensitive to national needs than the present machinery? And how, in any case, is the council to be composed? Will it consist of the government officials at present responsible as chief scientific advisors and heads of the research councils for spending money, in which case it will be yet another device for horse-trading and back-scratching? Or will it be independent of the existing machinery, in which case it is likely—however powerful the minister—to be ineffectual? The committee would have done its own public reputation a service if it had given some sign of thinking through these questions. To have resurrected the old proposal for a Minister of Research and Development is to have substituted a magic wand for serious consideration.

The poor quality of the committee's argument is best illustrated by its proposals for standardizing the nomenclature at present used to describe different kinds of research. Like many people in the past few months, the committee had plainly been bamboozled by the use of terms such as "tactical research" (a Daintonism) and "applied research" in the sense defined by Rothschild. Poor thing, why must people continually afflict the committee's sensibilities by redefining the different functions of research? The committee has a remedy which is in its simplicity absurd. Let the new council, which it proposes, devise standard definitions and, just to make sure that they will not change, register them with "international standard-making bodies such as OECD". Did not a single member of the Select Committee recognize that the names which have been used for the argument about Dainton and Rothschild are different from those familiar from previous arguments because the argument was different?

In the circumstances, it is no surprise that the committee has overlooked the opportunities which its inquiry has provided for a proper examination of the important questions raised by Rothschild. The first of the committee's reports accepts that the letting of research contracts by government departments to the research councils "may be important", especially in sharpening some of the work of the Agricultural and Medical Research Councils, but then goes on to ask for more detailed analysis and study. The point is, however, that nobody will be in a position to know how the system would function in the fields of basic research covered by the research councils until it has been tried. No amount of committee work can be a sufficient substitute for experiment, and nobody can anticipate at this stage how quickly the government departments can be properly equipped to formulate sensible proposals for contract research until one of them has been given the job of trying.

The underlying issue of accountability is dealt with in the committee's counter-proposals by a suggestion that



there should be quinquennial reviews of the work of the research councils by *ad hoc* committees of the Council on Science and Technology, with public reports to Parliament. One practical difficulty here, of course, is that no British minister, especially if he has a seat in the cabinet, will agree that reports from advisory committees should be published as of right. The chances are that what emerged would be as anodyne and as self-congratulatory as are the annual reports of the research councils at present. After all, it is remarkable but not surprising that in the whole argument about Rothschild, nobody has mentioned that the origin of the row lies in the poor organization of the Agricultural Research Council, now apparently openly acknowledged. If the committee really wants periodic reviews of the research councils' work, and that would be proper, why does it not resolve to undertake them on its own account?

The chief merit of the Rothschild recipe is that it would have put responsibility for a good deal of basic research in Britain where it properly belongs, in the government departments. And the truth is that under the British constitution, ministers in charge of government departments are at present accountable to Parliament not merely for their obvious executive functions but for their use of scientific research or, more commonly, for its neglect. One of the defects of the research council system is that it has allowed government departments to shelter behind the research councils. Especially if contracts let under the customer-contractor principle were published, it would at least be possible for government departments to be saddled with responsibility for what they do or neglect to do. If, in the process, they were compelled to equip themselves with competent scientific advice, that would be an extra benefit.

Housing Policy Muddle

THE British Government seems bent on perpetuating the muddle over housing policy which has bedevilled British housing policy for decades. Mr Peter Walker, one of the most pliable of ministers, responded last week to the complaints which there have been about the housing shortage with the announcement that an extra £80 million would be made available to local authorities for the purchase of land on which to build dwellings of all kinds. The immediate consequence will no doubt be that there will be far more outlets for the funds which the building societies have to spend. More agricultural land will disappear under concrete and bricks. The urban and suburban sprawl will spread still further. And then, perhaps even next year, money will be hard to come by again, the building societies will become reluctant lenders, and the housing market will embark on yet another of its periodic cycles of boom and bust. Especially now that Mr Walker prides himself on the title of Secretary of State for the Environment, and boasts of the way in which his post allows him to superintend the fashioning of the face of Britain as a whole, it would have been better if he had stood back a little from the problem and asked what the long-term objectives of housing policy should be.

The first thing to be said is that the present imbalance between the demand for housing and the supply could in principle be met in other ways than by increasing the

supply. Few people will deny that the British people are badly housed, but the present boom in the mortgage market is a flash in the pan. There might have been something to be said, on this occasion, for an attempt to redress the balance by making it harder for building societies to be easy lenders. Especially because Mr Walker's first claim on public and political attention stemmed from his success as a financial wizard, it is a surprise that he has apparently neglected this possibility.

The Department of the Environment should also have paid some attention to the question of where all the new houses will be built. Over the years, British governments have by all appearances been dedicated to the view that cities are unpleasant places. As a result, they have done their best to spread the bricks and concrete over the face of England and even of the Celtic fringe. The result is that even though close on a half of the population of the United Kingdom lives south-east of the infamous line from the Solent to the Wash, and does so by inclination, London is distinguished among metropolitan cities by its emptiness.

What is needed in Britain now is a resolution by the metropolitan authorities that the time has come for a radical revision of their previous creed of dispersion, and a recognition that it should be possible somehow to combine a policy for the improvement of the cities with a self-denying ordinance on the further exploitation of good agricultural land. In short, what Mr Walker should have decided is that the time has come when the great cities of the United Kingdom should have their arms twisted to agree that they and not the suburbs must grow. The objective should be to provide the British people with the best of all three accessible worlds—the improvement of the cities, the preservation of the countryside and the housing of the people.

100 Years Ago



At the meeting of the Royal Geographical Society held on Monday evening last, Sir Henry Rawlinson said that the opinion of the Council of the Society was favourable to the authenticity of the intelligence received by telegram respecting Dr. Livingstone. They had every reason to expect that Dr. Livingstone and Mr. Stanley would meet about the beginning of the year. But if there had been any discovery and relief, it was Dr. Livingstone that had discovered and relieved Mr. Stanley, and not Mr. Stanley who had discovered and relieved Dr. Livingstone; because Dr. Livingstone was in clover and Mr. Stanley was absolutely destitute. They knew by the last account that Mr. Stanley was without supplies, and he must have undergone much difficulty in getting to Ujiji; whereas this place was the head-quarters of Dr. Livingstone's supplies. He expected that they would have full letters in the course of a fortnight from Zanzibar, which would inform them on what was known about Dr. Livingstone and Mr. Stanley, and in the meantime he could only say that the telegram was credible.

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OLD WORLD

Select Committee Produces its First Report

THE Select Committee on Science and Technology recommended last week that no transfer of funds from the research councils to government departments be carried out until the result of full and detailed reviews of the resources for agricultural, medical and environmental research are available. The committee also recommends that there should be a Minister for Research and Development with his own department and vote and a seat in the cabinet (Cmnd. 237, HMSO, £0.42).

These revolutionary recommendations come after the select committee, under the chairmanship of Mr Airey Neave, had considered the evidence collected in twelve sessions since January, following the publication last November of the green paper *A Framework for Government Research and Development*. The green paper included the reports of Lord Rothschild on the organization and management of government research and development and the Council for Scientific Policy's report on the future of the research council system.

The select committee is unimpressed with Lord Rothschild's report although it points out that some of the criticism which has resounded through British scientific circles in recent months must be laid at the door of the government.

In particular the select committee says that a report carried out by one man "was bound to provoke debate" and it was wrong for the government to "introduce a consultative document by welcoming and endorsing Lord Rothschild's principal recommendations in advance of the wide public debate to which they referred in the green paper".

The select committee defends its recommendation that no transfer of funds be carried out by saying that the figures in table 4 of Lord Rothschild's report "can only be an arbitrary assessment of the sums which he [Lord Rothschild] believes should be transferred to the control of the departments".

The recommendation that there should be a Minister for Research and Development stems from the committee's conclusion that Britain should have a national policy for research and development. Mr Airey Neave said last week that at the moment the combined policies of the government departments do not collectively make up such a national policy. Mr Neave also said that he did not think that a national



Mr Airey Neave, chairman of the select committee. "Most important report which select committee has ever submitted to parliament".

policy should arise from departmental policies.

To aid the new minister, the select committee recommends the creation of a Council for Science and Technology with the new minister as its chairman. This committee would be a much stronger body than the present

Council for Scientific Policy which advises the Secretary of State for Education and Science and it would also be a much stronger body than the Board of the Research Councils recommended by the Council for Scientific Policy in its own report published in the green paper. The committee suggest that subcommittees of the council would deal with defence research and the research councils.

The select committee says that neither the Rothschild nor the Dainton reports "take enough account of the need to involve sections of society other than the scientific community and government departments in decisions about policies for science". The committee recommends that this be corrected by including representatives of industry, trade unions and other outside bodies on the subcommittee for the research councils. In particular the select committee sees the role of this committee as advising the Secretary of State for Education and Science "in the broad context of the national effort" on policies affecting the research councils as a whole.

Part of the report is concerned with the semantics of definitions of research. Mr Airey Neave and his committee consider that a great deal of time has been wasted in recent months because of inadequate definitions of research classes and the use of different definitions,

Analysis of Research

THE Agricultural, Medical and Natural Environment Research Councils have provided the following classification of their own research efforts divided into categories as suggested by the Select Committee on Science and Technology. The categories are:— Applied: research and development with a practical application as its objective. Oriented

strategic: background research thought likely to be necessary in order to achieve a specified objective. Basic strategic: background investigation which may or may not help in reaching one or a number of applied objectives. Basic: research and development undertaken for its intrinsic merit with no useful application in mind.

Table. Analysis of Research Carried out by Research Councils as a Percentage of Total

	Applied*	Oriented strategic	Basic strategic	Basic
ARC	21 (77)	18	39	22
MRC	8 (25)	41	51	—†
NERC	4 (50)	12	54	30

*Figures in parentheses are Lord Rothschild's assessment of the applied research carried out by the research councils.

†The MRC did not quote a figure for basic research because according to the council "no research is financed unless the council hopes that it will contribute towards its objectives".

resulting in a series of misinterpretations. To avoid any such controversy in the future the committee recommends that the Council for Science and Technology should study the problem so that standard definitions can be arrived at. With entry to the European Economic Community drawing near the committee considers that standard definitions should be drawn up by bodies such as the OECD.

The report is the first of three to be put out by the select committee in the next few months. The remaining two reports will be devoted to the research establishments of the Department of Trade and Industry and the civil research and development activities of the United Kingdom Atomic Energy Authority.

Mr Neave also said that it was imperative that the "scientific community no longer be kept in suspense" and that the reviews his committee recommend be carried out as soon as possible.

One of the suggestions of the select committee that is bound to meet with universal approval within the scientific community is that the government should implement more quickly the Fulton proposals for unified grading within the civil service beneath under-secretary level. The select committee quote Lord Rothschild as saying that the immobility of the scientific civil service is a "major national problem". Mr Neave said last week that the select committee is not "encouraged by the attitude of the permanent secretaries as to the fulfilling of the Fulton proposals".

The select committee expresses concern that the planned decrease in the rate of growth of the British science budget for the next two years might have a deleterious effect on university science. The committee adds that it would be "gravely concerned at any measures which would impair the close relationship of universities with the research councils", further "a principal objective of a national science policy must be to ensure that university administrations, budgets, laboratories and standards of excellence are maintained at the highest levels possible".

The select committee also recommends that both research councils and departments formulate their research on a five year basis which would be subject to scrutiny by the Minister for Research and Development and the Council for Science and Technology.

Whereas the committee feels that the research councils should present their annual reports in such a way that more information is given about the way in which they spend their money, it is more critical of the government departments, some of which publish no annual reports on research and development at all. The committee recommends that all departments should in future publish annual reports in a standard form.

The following are the detailed recommendations of the select committee on science and technology.

1. As a general rule the consultative nature of the recommendations contained in a green paper should not be impaired and public discussion should not be prejudiced by government statements of policy in advance, and future reviews of government research and development should include the taking of evidence from all interested parties and that as much of that evidence as possible should be published.

2. There should be a Minister for Research and Development with his own Vote, who should be a member of the Cabinet with statutory power to examine and approve all government research and development.

3. Government departments should formulate their research and development programmes on a rolling five-year basis and submit them to the Minister for Research and Development for his approval.

4. A statutory Council for Science and Technology should be set up consisting of not less than twelve persons of which the Minister for Research and Development should be the chairman. Its functions should be:

(a) to advise on the formulation of policy and priorities for expenditure on civil and defence research and development;

(b) to assess the likely or potential needs of the community in relation to developments in science and technology;

(c) to anticipate and to provide the necessary research and development to deal with any hazards which may threaten the community; and

(d) to commission its own studies whenever necessary from departments, research councils, industry or university groups.

5. This council should be empowered to set up committees to deal with particular areas of research and development for specific problems. There should be a committee or committees dealing with Defence Research and a Committee for the Research Councils.

6. The Minister for Research and Development should make a full annual report to Parliament.

7. The proposed Council for Science and Technology should commission a study of the problem and seek to draw up standard definitions for research and development.

8. The Council for Science and Technology should collaborate closely with international standard-making bodies such as OECD and should take account of foreign practice in drawing up standard definitions for the United Kingdom.

9. The membership of the research councils should be reconstituted to ensure a wider representation of government departments, industry, trade unions, the universities and the professional staff associations.

10. The research councils should re-examine the form of their annual reports to ensure that the fullest information is given as to how their financial resources have been allocated and are being expended.

11. Any re-allocation of responsibilities for programmes of research between departments and research councils should be made in the light of joint reviews of the current programmes and needs. Particular attention should be paid to the investigation of potential hazards to life and health.

12. The budgets of each research council should be increased to permit an improved headquarters staff, able to provide guidance and information to users and closer control of programmes.

13. The work of each research council should be examined every five years by an ad hoc committee of the Council for Science and Technology and a quinquennial report should be made to Parliament.

14. The government in its white paper should state clearly what the role of the research councils should be in the whole field of research and development.

15. The proposed Council for Science and Technology should set up a Committee for the Research Councils to take the place of the Council for Scientific Policy.

16. The government should make faster progress in implementing the Fulton Report.

17. The Council for Science and Technology should commission reviews by Departments and the Medical Research Council, the Agricultural Research Council and the Natural Environment Research Council of the resources for agricultural, medical and environmental research taking account of: (i) the total resources available for research and development in these fields; (ii) the need to link research programmes with departmental objectives. We also recommend that no transfer of funds should be made until these reviews have been completed.

18. All government departments with research and development activities should publish in a standard form annual reports on these activities. The reports should include: (i) statistics explaining the size of their total research and development budget; (ii) the amounts and objectives of research expenditure on separate projects; (iii) progress reports on projects; (iv) assessment of the results of former research and development work; (v) machinery for dialogue with potential users, customers, contractors.

SOVIET SCIENCE

Polluted Rivers

from our Soviet Correspondent

THE recent industrial expansion in the Ural area has, inevitably, led to a major pollution problem in the Volga and Ural rivers. The problem has now reached such proportions that anti-pollution measures for these rivers became the subject matter of a recent resolution of the Soviet Government, and became headline news in *Pravda*.

Starting with a minor note of self-congratulation, the resolution states that in the past five years, 670 purification plants have been installed on these rivers, with a total capacity of over 3 million m³ a day. Many manufacturing plants have also introduced water-free processes or waste-recovery units.

The capacity of recirculatory water systems has increased to some 70 million m³ a day, and the discharge into the rivers of such pollutants as petroleum products, copper, zinc and phenols has been "considerably" reduced. Nevertheless, the very wording of this claim seems to suggest that the amount of such pollutants entering the rivers is still by no means negligible.

Immediate plans, under the new resolution, also underline the magnitude of the problem. 421 major manufacturing enterprises are to be equipped with purification plants by 1975 at a cost of 700 million roubles. Another 300 million roubles are allotted to urban sewage and purification plants in 15 towns on the Volga and its tributary, the Kama. Looking forward to the next 5 year plan (1976-80) it is claimed that by the end of the decade discharge of unpurified industrial wastes and sewage into these rivers will have been "totally stopped".

As an immediate anti-pollution measure, the Ministry of Fisheries and Ministry of River Navigation of the USSR are to equip the ships using the Volga and Ural rivers with "devices and systems for collecting waters polluted by urban wastes and bilges". At the same time collecting devices for petroleum wastes and other liquid and solid pollutants are to be installed in the river ports and shipping basins of these rivers. The resolution, however, does not indicate the nature of the devices to be used, either for the ships or the port installations.

The Ministry of Irrigation and Water Economy and the industrial ministries are also to undertake projects aimed, not only at cutting pollution of the Volga, but also at eliminating wastage of the river water by industry. Although the Volga is the largest river of the Soviet Union, it is now barely able to maintain a sufficient water supply for all the demands made upon it by indus-

try and irrigation. Thus the plans, first envisaged in the 1930s, of diverting the waters of the north-flowing Siberian rivers to feed the Volga and its tributaries are now being implemented. Since the major drought of the 1930s, when the annual influx of water from the Volga into the Caspian fell from 250 to 170-190 km³ per year, the water-level of the Caspian has been falling and the salinity rising with a consequent threat to the caviare fisheries, already threatened by the expansion of offshore oil-drilling.

METRICATION

To Keep the Ball Rolling

THE impetus must be restored to the changeover to metric units in Britain or the national economy and individual businesses will be faced with uncertainties which could be "seriously damaging" according to the Metrication Board's third report published last week (*Report of the Metrication Board, 1971*, HMSO, £0.90). The report goes on "only the government can give the positive lead which is essential to restore confidence and recover the buoyancy and energy which is necessary if metrication is to resume its advance on a broad front as a coherent national policy".

But in spite of the hiatus caused by the non-appearance of the govern-

ment's white paper on metrication until February 1972, the metrication board reports that progress in 1971 was "broadly satisfactory". The construction industry kept to its timetable of complete metrication by 1973, but the rate of change in the engineering industry was not as rapid as in previous years. Progress in the fuel and power industries continued to be satisfactory, and the metrication of overseas freight went ahead well, although inland freight made fewer changes. The board also feels that the major part of the work of the Steering Committee for Education and Industrial Training has been accomplished.

In conclusion the board says that "it continues to be our view that, given a firm lead by the government, Britain can substantially accomplish the changeover to metric by 1975".

Lord Ritchie-Calder, retiring chairman of the Metrication Board, said last week that the white paper had removed uncertainties about the government's metrication policy and had defined the task ahead. Lord Orr-Ewing, the board's new chairman, said that the rate of metric change must be speeded up; if the United States metric conversion bill becomes law, 99 per cent of Britain's exports and 99 per cent of world trade will be with countries that have chosen metric. "It is unthinkable that Britain should lag behind and become an isolated imperial island in a totally metric sea."

LONDON ZOO

New Home for Primates

WITH the opening last week of the Michael Sobell pavilions for apes and monkeys, named after its benefactor, at the Zoological Society of London's gardens in Regent's Park, a new high standard for the husbandry and exhibition of these animals in urban zoos is set. The old scene of lonely animals in barred, bare cages is swept away and the zoo now offers a select thirteen species of the larger monkeys and apes the chance of domesticity and family life, and the next best thing to trees.

The species of primate which have been chosen to spend their days in such luxury include the mandrill, pig tailed macaque, colubus, orang utan, chimpanzee and lowland gorilla. These animals are housed in family groups or colonies in five pavilions or den blocks and each animal has the new found advantage of being able to move freely between its inside den and an outside enclosure.

The orang-utan shown here is taking exercise on the tubular steel space frame which has been fitted in each outside enclosure to provide the animals with horizontal routes high above ground so

that arboreal species can move about as they would in trees in their natural habitat. The animals can also climb on the tensioned wire mesh which surrounds the enclosures—and which also keeps the public at bay—and there are more climbing structures inside the dens. Spectators are clearly going to enjoy the new pavilions. Time will tell whether the arrangements will encourage the animals to breed.



Orang-utan in the new Michael Sobell pavilions for apes and monkeys at the London zoo.

NEW WORLD

NIH to Lose Control of Vaccines

by our Washington Correspondent

THE Division of Biologics Standards, an agency of the federal government charged with the task of regulating the safety and potency of biological products, has for most of the past sixteen years carried out its job in almost total obscurity. But, during the past few months, a series of bruising public and private inquiries into the way it runs its affairs have lifted the DBS into the public eye and left in tatters any reputation it may once have had. The result of this sudden and devastating public exposure is that part of the DBS is to be transferred from the National Institutes of Health, where it now resides, to the Food and Drug Administration, and that a search is under way for a successor to the present director of the agency. These moves were announced last week by Elliot L. Richardson, Secretary of Health, Education and Welfare, who was giving evidence to Senator Ribicoff's Senate Subcommittee on Executive Reorganization.

The scenario which has led to the transfer of some of the DBS's functions to the FDA was sparked off chiefly by Dr J. Anthony Morris, a DBS scientist who accused the DBS leadership of harassing him because of his belief that commercial influenza vaccines are largely ineffective. A committee of scientists charged with evaluating Morris's complaints has upheld them, another internal NIH committee has since found severe personnel problems in the DBS which have interfered with the way in which it operates, the General Accounting Office has come up with the much publicized finding that the DBS has been releasing subpotent influenza vaccines to the market, and finally, Senator Ribicoff's subcommittee has recently held a series of hearings during which many complaints about the DBS have been given a public airing. In addition, Morris and James Turner, his attorney who carried out a study of the Food and Drug Administration for Ralph Nader, made public last year a series of remarkable charges against the DBS, and these have since been the subject of charges and counter charges by individuals and committees of the National Institutes of Health.

Faced with this damaging public spotlight on the skeletons in the DBS cupboard, the Department of Health, Education and Welfare has been forced to take some public action to help stave off criticism. Apart from appointing committees to review the charges of scientific mismanagement levelled by

Morris and Turner, its first move came in February this year, when it gave the DBS the formal power to prevent ineffective vaccines from reaching the market. The DBS was set up in 1956 and charged with the task of making sure that biological products which reach the market are both safe and potent, but there has been considerable confusion about whether or not the agency has the power to make sure that the biological products it licenses actually do the jobs that they are designed for. (Potency and efficacy are not necessarily synonymous.)

Officials of the DBS have claimed that the agency has never had the power to regulate the efficacy of vaccines, because such a provision was specifically deleted from the Public Health Service Act. The general council of HEW, on the other hand, has claimed that the agency could be given such power under the terms of the Food and Drug Act, and that is essentially what took place in February.

That action was, however, insufficient to placate the agency's critics who maintain that the DBS's trouble stems from personnel problems and from attitudes that have been built up over the agency's lifetime. Perhaps the most crushing charge levelled at the DBS by Morris and Turner is that the agency's leadership actively discourages research by DBS scientists which may question the efficacy or safety of a vaccine. The basis for the complaint is that the DBS has a dual role as both a promoter and a regulator of vaccines and that this clouds the attitudes of some DBS officials to potentially damaging research.

Last week's announcement that the regulatory functions of DBS are to be split off and transferred to the Food and Drug Administration may go some way towards meeting the criticism that the agency suffers from a conflict of interest, but a critical question is how much of the agency's functions will be transferred, and what will become of the research activities which will remain at NIH. The hearings shed little light on these considerations, for both Mr Richardson and Dr Robert Berliner, Deputy NIH Director for Science, said that the details have not yet been worked out. Richardson also confessed that the planned transfer is taking place partly to head off the move towards setting up a consumer safety administration outside the Department of Health, Education and Welfare, that is embodied in bills before Ribicoff's subcommittee.

A clue to the likely extent of the transfer is, however, contained in an internal memorandum from Dr Robert Q. Marston, Director of NIH, to Dr Merlin K. DuVal, Assistant Secretary for Health and Scientific Affairs at HEW. Dated one day before Richardson announced the transfer, the memorandum outlines a scheme for transferring only about 10 per cent of the agency's staff and budget to the Food and Drug Administration, while leaving research and development of vaccines within NIH. Marston also expressed his preference for keeping all the present DBS functions within NIH, and pointed out that it is difficult to separate purely research activities from the agency's regulatory functions.

Marston's suggested transfer would also leave all of the present DBS laboratories within NIH, where they would be "integrated into the NIH biomedical research program". Whether this means that the DBS would be completely dissolved and absorbed by other institutes of NIH is open to question, but its most immediate effect is to throw a spanner into the workings of a committee which has been looking for a successor to the present director of the DBS. Richardson said last week that it will now be the responsibility of the Commissioner of the Food and Drug Administration to find a new head for vaccine regulations, and Berliner said that the committee that is now sifting through possible candidates for the post of Director of DBS will probably halt its activities until it is decided whether there will be sufficient operations left at NIH for a separate division.

What difference, in any case, will it make when the DBS's regulatory functions are transferred to the FDA? Perhaps the chief change will be that the agency will no longer be able to enjoy the obscurity that it has had in the past. For one thing, Ribicoff's subcommittee will be able to exercise oversight over its affairs, and his companion committee in the House of Representatives, a committee chaired by Rep. L. H. Fountain, will similarly be able to spotlight any shortcomings in its operations. But critics of the present arrangement also point out that the transfer will provide a complete break with the attitudes that they claim have dogged the division in the past. The agency's critics also claim that a change of leadership in the DBS will let some fresh air into the rigid authoritarian structure that has been built up within the agency itself.

The present director of the DBS, Dr Roderick Murray, was recently criti-

cized by a management study committee appointed by the NIH to look into allegations of personnel problems in the division. The committee's report said that Dr Murray delays making decisions and that his managerial style is over-cautious—a trait which has caused the DBS "to sacrifice some dynamism and aggressiveness". Dr Murray is due to retire in about 18 months time, but it became clear from the hearings last week that he will be replaced as soon as a successor is found, assuming that the scale of the DBS's activities will be sufficient to maintain a separate division within NIH.

Before the DBS is transferred to the Food and Drug Administration, it will clearly be necessary to work out suitable arrangements for bringing the results of the research conducted at NIH to the notice and use of those who will be regulating biological products in the FDA. One of the arguments for maintaining both research and regulation in the same division in the past has been that research results should be more easily applied in, for example, developing and using more sensitive safety and efficacy tests. It would be a sad mistake if, in attempting to tighten up the DBS's regulatory activities, the proposed transfer makes the regulators less responsive to changes in the science of biological products. Institutional attitudes seem to have hampered such transfer in the past and there is a fear that institutional demarcations could do so in the future.

MANPOWER

Unemployed Chemists

by our Washington Correspondent

WHEN this year's crop of chemistry graduates leaves the universities it will be faced with a job market that is even tighter than it was a year ago. According to a survey carried out by the American Chemical Society, unemployment among chemists and chemical engineers is running at about 3.0 per cent, compared with 2.7 per cent last year. And, more ominously for new graduates, the jobless rate among chemists under the age of 25 has more

than tripled in the past year; it now stands at 23.7 per cent according to the survey.

Based on returns to a questionnaire mailed to members of the American Chemical Society in February, the survey indicates that there are some 5,600 chemists of all types out of work and another 7,800 employed part time or in occupations for which their professional training is not required. With some 13,000 more chemists entering the job market this summer, there could be as many as 23,000 chasing the available jobs but, according to the American Chemical Society, the job market will expand to accommodate only about 11,000.

The chief culprit in the declining job market for chemists seems to be the Federal government, and in particular the drop in federal expenditure on research and development which set in during the late 1960s. The survey shows, for example, that some 46.3 per cent of those who were unemployed had previously been funded by the government, while government funds supported only 24.9 per cent of all the respondents to the questionnaire. Moreover, while only 4.3 per cent of PhD chemists are employed in defense industries, 25.6 per cent of the pool of unemployed PhD chemists came from that source.

Other findings in the survey include:

- Women chemists are hit harder than men by the tight job market—although women make up only 6.6 per cent of the total sample, they account for 15.8 per cent of the unemployed.

- There is surprisingly little difference between the unemployment rate for first degree graduates and that for PhDs. A total of 3.3 per cent of those holding a BS degree reported that they were out of work, compared with 3.5 per cent of those holding a master degree and 2.5 per cent of PhDs. If these totals are broken down according to sex, however, it turns out that 7.2 per cent of women who hold a BS degree, 6.9 per cent of those who have a masters and 7.6 per cent of those with a PhD are out of work, compared with 2.7, 2.9 and 1.9

per cent respectively of the men.

- Although the unemployment rate for chemists under the age of 25 rose from 7.0 per cent in 1971 to 23.7 per cent this year, the unemployment rate for chemists aged 36 to 50 has actually improved fractionally. But the position for all other age groups is worse this year than it was last.

- More than half the sample of unemployed chemists had been out of work for more than three months.

The survey is available from the American Chemical Society, and it is published in part in the current issue of *Chemical and Engineering News*.

DETERGENTS

NTA Still in Doubt

by our Washington Correspondent

IN the late 1960s, when detergent manufacturers were prodded by the rising chorus of concern about the environment to seek alternatives to phosphates for their products, they placed their hopes in a chemical called nitrilotriacetic acid (NTA). At first, NTA seemed to fit the bill admirably. It is even better than phosphates in its ability to combine with metals to prevent them from interfering with the action of the detergent, it is biodegradable and, unlike phosphates, contributes little nutrient to the surface waters.

But the new washday miracle soon turned out to be less of a great white hope than it seemed at first, for in December 1970, detergent manufacturers agreed to take it out of their products because of its possible risk to public health. And last week the Department of Health, Education and Welfare released a report prepared by an independent scientific committee whose conclusions prompted Dr Merlin K. DuVal, Assistant Secretary for Health and Scientific Affairs, to announce that his department will continue to oppose use of the additive in detergents.

The first doubts about the safety of NTA rested chiefly on a study conducted by scientists from Procter and Gamble who found an increase in the incidence of tumours in rats fed moderate doses of the additive for two years, and there were also suggestions that NTA may be mutagenic. Moreover, Dr Samuel Epstein, then at Boston Children's Hospital, suggested that intermediate products formed when NTA undergoes bacterial breakdown in sewage plants may combine with nitrites from the environment to form carcinogenic nitroso derivatives. And there have also been some fears that the very property which makes NTA useful in detergents—its ability to form complexes with metals—may cause it to put into solution metals such as cadmium and mercury in the environment, thereby

Table 1. Employment Status of ACS Members

	1971		1972		Total Chemical Population
	Total Sample	% of Sample	Total Sample	% of Sample	
Full-Time Employed	24,105	88.2%	22,462	88.0%	164,600
Unemployed	731	2.7	777	3.0	5,600
Temporarily/Part-time Employed	612	2.2	386	1.5	2,800
Sub-professional Employment	667	2.4	687	2.7	5,000
Post-doctoral/Fellowship	442	1.6	513	2.0	3,700
Retired, seeking employment	—	—	96	0.4	700
Retired, not seeking employment	698*	2.6*	564	2.2	4,100
No Report	70	0.3	54	0.2	400
TOTAL	27,325	100.0	25,539	100.0	—

* Includes those retired but seeking employment.

increasing the exposure of animals, including man, to their toxic effects. The report published last week, which was prepared under the chairmanship of Dr L. A. Woods of the University of Virginia, refutes the last two of these suggestions, but says that there are insufficient data available to decide whether or not NTA is either carcinogenic or mutagenic.

The Department of Health, Education and Welfare, which last year caused considerable confusion and consternation when three of its top officials recommended that phosphate detergents should be used in preference to those containing caustic substitutes, is therefore biding its time by suggesting that manufacturers stick to their voluntary ban on NTA until sufficient evidence is available to declare it safe for use.

The committee's conclusions, based on a survey of the literature and on the experience of heavy use of NTA in Canada and Sweden, are summed up in the report with the view that apart from "major reservations" about its carcinogenicity and mutagenicity, "NTA is not likely to be toxic to man in levels encountered in daily use". Studies of its acute toxicity have shown it to be about as toxic as the polyphosphates which it would replace in detergents, and "eye irritation, percutaneous toxicity, primary irritation and sensitization studies are essentially negative". As for longer term chronic toxicity studies, the report suggests that changes have so far been limited to only one organ, the kidney, with vacuolar lesions found in the tubules when NTA is fed to rats at about 0.5 per cent of the daily diet.

Such studies are, however, "insufficient to define a dose level unaccompanied by toxicity", and long term, low exposure hazards to man "cannot be confidently evaluated from examination of the available data", the committee suggests. Similarly, although submamalian tests fail to show any increase in gene mutations due to NTA, there have been conflicting results from studies of the effect of NTA on chromosome integrity. There is therefore room for doubt about the mutagenic potential of the additive. The committee also has reservations about the design and conduct of the study of Procter and Gamble scientists, which came up with the possible finding that NTA is carcinogenic. The strain of rats used in the study, for example, had a high incidence of spontaneous mammary tumours, kidney disease and early death, but nevertheless the doubts cannot be resolved unless further studies are carried out.

The committee believes that NTA would pose little threat to the environment chiefly because it is degraded by sewage plants and diluted to such an extent that its ability to form complexes

with metals is small, and estimates based on the maximum probable use of NTA indicate that it would increase the level of nitrate in lakes and streams by less than 1 per cent. Phosphates from detergents, on the other hand, are believed to contribute as much as 60 per cent of the phosphorus input into some waters and phosphorus is widely claimed to be the limiting nutrient for the growth of algae.

The report also points out that in Canada, where NTA is widely used, tap-water contains on average 1 or 2 parts per billion of NTA, and that the average daily intake of the additive is about 100 µg. This level of intake is several orders of magnitude less than those used for long term feeding studies in rats.

NATIONAL CANCER INSTITUTE

New Hand on the Helm

PRESIDENT NIXON last week formally announced that he has appointed Dr Frank J. Rauscher jun. to replace Dr Carl Baker as director of the National Cancer Institute. The widely predicted appointment puts Rauscher at the head of the much publicized campaign against cancer that was launched in the bill which was passed by Congress last December.

Although the directorship of the institute is technically a new position since it is a Presidential appointment, the job will not be greatly different from that performed by Dr Baker. Rauscher, aged 40, is at present Scientific Director for Etiology at the National Cancer Institute. A microbiologist who received his training at Rutgers University, he is best known for his discovery of the Rauscher leukaemia virus. Dr Baker will be staying on at the National Institutes of Health as "Special Assistant to the Director for Technology Implementation", a position created for him which is at present no less vague than its title.

One thing that Rauscher can be sure of is that his budget will increase steadily in the next few years. President Nixon, for example, recently sent to Congress a request for an extra \$40 million for the institute in 1973 for construction of new facilities and for providing fellowship and training grants.

If Congress accepts the supplementary appropriation would give the NCI \$378 million in 1973—a 100 per cent increase since 1970.

Short Notes

Sickle Cell Anaemia

CONGRESS has finally passed a bill which authorizes \$115 million to be spent over the next three years on research, screening and counselling programmes designed to combat sickle cell anaemia. The bill now goes to President Nixon for his signature, and no opposition by the Administration is expected. The funding proposed in the bill would represent a huge increase compared to present expenditures, which are running at less than \$10 million a year, and it would be earmarked chiefly for the setting up of new centres to offer screening and genetic counselling services. A hereditary disease that afflicts some 2 million, chiefly black, Americans, sickle cell anaemia is the product of a gene mutation believed to have occurred in Africa several centuries ago as a protection from malaria. The recessive gene mutation is carried by about 10 per cent of the black population in the United States, and the thrust of the programme outlined in the bill is to pinpoint carriers of the trait and to provide counselling services for those couples that run the risk of bearing children with the disease. The disease itself is characterized by painful "crises" which occur when red blood corpuscles form themselves into the characteristic sickle cell shape, blocking blood vessels and restricting blood flow to the extremities.

OU for US

BRITAIN'S Open University is scheduled to make its debut on North American campuses later this year. Rutgers University and three other institutions, one of which will probably be the University of California, are planning to experiment with the Open University idea in the 1972-73 academic session. A total of about 800 students will take part in the trial run, and the venture is being financed by a grant from the Carnegie Corporation of New York. Britain's Open University should have a welcome financial stake in the project, for it will probably supply teaching materials and systems information to the participating institutions.

Lunar Science in Arizona

THE Lunar and Planetary Laboratory of the University of Arizona will soon have its own undergraduate students. Since 1960, the institution has been entirely a research facility, although a few graduate students from other departments in the university have carried out their PhD studies there. But last week it was designated as the Department of Lunar and Planetary Sciences, a status which allows undergraduate and graduate courses to be taught there.

NEWS AND VIEWS

Complete Sequence of Phage Gene

PERSEVERANCE is perhaps the chief quality demanded of anybody intent today on determining the sequence of an RNA molecule which can be obtained pure and in reasonably large amounts, and Fiers and his associates, Min Jou, Haegeman and Ysebaert, in Ghent clearly possess it in full measure. As they report on page 82 of this issue of *Nature*, using conventional RNA sequencing techniques, together with one or two refinements they have themselves pioneered, they have determined the complete sequence of the coat protein cistron of coliphage MS2 RNA. This is, of course, the first time that the complete sequence of any gene which codes for a protein has been determined and the work of the Belgian group is nothing if not a *tour de force* which should greatly encourage others now attempting to sequence the messenger RNAs which specify haemoglobin, immunoglobulins, insect silk and other proteins.

The RNA of the RNA coliphages, which serves both as a messenger and as a genome, has for the past several years been a happy hunting ground for RNA sequencers, not least because it can be easily obtained in large amounts and labelled to high specific activities. Long fragments of coliphage R17 RNA, including parts of the coat protein cistron, the initiation sites of all three R17 cistrons, and the termination site of the coat protein cistron, have, for example, been sequenced by Sanger and his collaborators at Cambridge. Large tracts of phage Q β RNA have been analysed by Weissman and Hindley and their collaborators, and Fiers and his colleagues have reported the base sequences of several fragments of MS2 RNA.

Armed with all this accumulated information, and knowing the amino-acid sequence of the MS2 phage coat protein, Fiers and his colleagues slogged on with the task of analysing ever larger overlapping fragments of the MS2 coat protein cistron obtained by partial digestion of MS2 RNA. As they report, they ended up with the sequences of four long but non-overlapping fragments which include the whole of the coat protein cistron and tracts extending beyond that cistron at both 5' and 3' ends. They were then able to order these four fragments by referring to the recently determined amino-acid sequence, and therefore possible codon sequences, of MS2 coat protein. This coat protein, it turns out, is not as was previously thought identical to R17 coat protein but differs at three positions (positions 11, 12 and 17).

Apart from confirming in the most direct way possible the codon assignments that have been deduced from experiments with *in vitro* systems (forty-nine codons are used to specify MS2 coat protein), and explaining the specificity of the mutagenesis of the RNA coliphages, the sequence of the MS2 coat protein has several interesting features. First, it is clear that the sequences of non-translated bases which precede the initiating AUG codons at the beginning of the coat protein and RNA replicase subunit cistrons of the RNA phages have been conserved during evolution, for these ribosome binding sites in MS2, R17 and f2 RNAs are virtually identical. How initiating AUG codons which specify formylmethionine are distinguished by the translation machinery of *Escherichia*

coli from internal AUG codons which specify methionine remains, however, as much a mystery as ever it was, for the sequences of the ribosome binding sites of the various cistrons of these three phages do not fall into any obvious and common pattern.

Second, there is ample evidence indicating that the RNA of the RNA coliphages has a complex secondary and tertiary structure. The mere fact that MS2 and related RNAs are not digested at random by nucleases indicates that certain parts of the molecule are exposed whereas others are protected from nucleolytic attack, and Fiers *et al.* have, by comparing the length of fragments of the MS2 coat protein cistron with their electrophoretic mobility, obtained direct evidence of such secondary structure. They have therefore produced a model of the secondary structure of the MS2 coat protein gene by drawing out the sequence in such a way as to maximize base pairing and generate what is probably the thermodynamically most stable possible configuration. This favoured conformation they aptly describe as the "flower" structure model, for it comprises a long double stranded "stem" surmounted by a set of radiating double stranded hairpin loops, the "petals".

There seems little doubt that the general outline, if not every precise detail, of this model is correct and in reality this part of the MS2 RNA is probably further folded on itself to give some even more complex three dimensional structure. As Fiers *et al.* comment, during evolution selection for a particular secondary structure may have dictated the occurrence of particular degenerate codons in this sequence and the need to conserve the structure of the molecule may explain why only forty-nine codons are used.

Finally, the "flower" model of the secondary structure of the MS2 coat protein cistron neatly explains the phenomenon of genetic polarity in the RNA coliphages. Mutant phage with an amber (AUG) codon in place of the glutamine (CAG) codon at position six in the coat protein not only fail to specify coat protein but also fail to make RNA replicase subunits. By contrast mutant phage with an amber codon in place of the glutamine codon at position 50 in the coat protein, although failing to make coat protein, do make RNA replicase subunits.

From such genetic data it was concluded that the translation of the cistron specifying the RNA replicase subunit, which immediately follows the coat protein cistron in the phage RNA, depends on ribosomes translating the coat protein cistron between codons six and fifty; the idea was that the ribosome binding site of the replicase cistron is somehow made inaccessible to ribosomes by being hydrogen bonded in some secondary structure to part of the coat protein cistron between these two codons and that only after ribosomes have translated this region of the coat protein cistron and destroyed the secondary structure is the binding site of the replicase cistron made accessible. Sure enough in the "flower" model of the secondary structure of the coat protein cistron, derived, remember, from considerations of thermodynamic stability rather than genetic polarity, a stretch of RNA containing

the initiation site of replicase cistron is base paired in the "stem" with the RNA which specifies amino-acids 24-32 of the coat protein cistron.

If there is one chief conclusion to be drawn from the base sequence of the MS2 coat protein cistron it is surely that the RNA of these coliphages, which serves as both messenger and genome, is a highly struc-

tured molecule, a large proportion of which assumes double helical hairpin conformations. To what extent such structures are general features of messenger RNA rather than specific features of an RNA which as well as being translated must self replicate and assume a shape which allows it to be packaged within a small protein capsid will only become apparent

when other non-viral messengers have been analysed.

Several groups are, of course, already tackling the analysis of the sequence of such messengers and by the time they have completed their work Fiers and his colleagues may well have sequenced the complete MS2 RNA molecule.—From our Cell Biology Correspondent.

Interspecific Transfer of Nitrogen Fixation Genes

THE high yielding strains of rice, wheat and other cereals which have been introduced during the past few years into many Asian countries and have been widely heralded as the vanguard of a "Green Revolution" have one serious drawback; they need, to yield well, to be fed large amounts of nitrogenous fertilizers. And quite apart from any qualms there may be about the hazards of the excessive use of such fertilizers, this requirement places an extra, and in the long term perhaps insupportable, burden on the economies of nations barely holding their own in the fight to feed their populations.

If the plant geneticists could select high yielding cereals which require little in the way of nitrogenous fertilizers—perhaps by engineering strains with the inherited capacity to fix atmospheric nitrogen in sufficient amounts to meet their own needs—it might, of course, be possible to break out of the vicious circle. And although it would be totally wrong and misleading even to hint that such cereals are on the distant horizon let alone just around the corner, by investigating the genetics of nitrogen fixation in bacteria Professor John Postgate and his colleagues at the ARC Unit of Nitrogen Fixation at the University of Sussex, and other groups in this field, are at least laying the foundations from which the plant breeders may ultimately be able to build high yielding, nitrogen-fixing strains of cereals.

Having already successfully transferred the nitrogen fixation genes (*nif*) from a strain of *Klebsiella pneumoniae*, a bacterium which under anaerobic conditions can fix nitrogen, to a second strain of this species which lacks this ability (*Nature*, **234**, 47; 1971), Postgate and his colleague Dixon now report on page 102 of

this issue of *Nature* their successful transfer of the structural and regulatory *nif* genes from *K. pneumoniae* to a strain of the common gut bacterium *Escherichia coli* (*E. coli* C).

Although the precise chemistry of the nitrogenases made in the recipient *E. coli* cells and the nature and origin of the electron transfer proteins which allow the *E. coli* to fix nitrogen have yet to be elucidated, the transfer of functional *nif* genes from one bacterial species to another in a different genus is a considerable and exciting achievement. In what state the *K. pneumoniae* *nif* genes are stably associated with the *E. coli* cells also has yet to be determined; it would be no great surprise, however, if they were found to persist as a small closed circular piece of DNA independent of the *E. coli* chromosome and if that is the case it may be possible to get the *nif* genes into a plasmid state which would in effect render them infectious. Such a *nif* gene plasmid would be extremely useful, for it would facilitate both attempts to transfer these genes to other species and attempts physically to isolate them. And, once physically isolated, the way is open to investigations of the molecular biology of the *nif* genes in cell free systems.

The success of Dixon and Postgate's experiments stems in large part from their shrewd choice of the recipient strain, *E. coli* C, which lacks the so-called restriction and modification enzymes that are present in most other strains of *E. coli* and no doubt most other species of bacteria. Restriction nucleases can recognize, by virtue of the pattern of modified bases in a DNA, any foreign DNA and degrade it, and modification enzymes mark, by base modification, the DNA replicated in a cell as DNA belonging to that cell which should not be de-

graded. In short, the restriction and modification enzymes provide a cell with the mechanism for distinguishing foreign DNA from its own DNA and degrading the former and they are obviously a strong barrier against the interspecific transfer of genes.

By selecting *E. coli* C cells as recipients, Dixon and Postgate effectively short-circuited this obstacle and gave the *nif* genes an environment in which they would not be immediately recognized as foreign and degraded. Transfer of these genes to other strains and species of bacteria and to cells of higher plants, which if they do not have restriction and modification enzymes identical to those in bacteria will no doubt be found to possess other mechanisms for dealing with foreign DNA, inevitably will be a far more difficult task. But, great as the obstacles may be, the incentive, ultimately the introduction of nitrogen fixing genes into commercially important plants, is greater.—From a Correspondent.

MARS

Recent Mariner Results

from a Correspondent

ON April 24, at the annual meeting of the US National Academy of Sciences in Washington, a small symposium, convened by Dr William Pickering, director of the Jet Propulsion Laboratory, Pasadena, was devoted to recent findings on the planet Mars obtained by Mariner 9—the first man-made object to orbit another planet. The speakers presented a representative but by no means exhaustive sampling of the preliminary data.

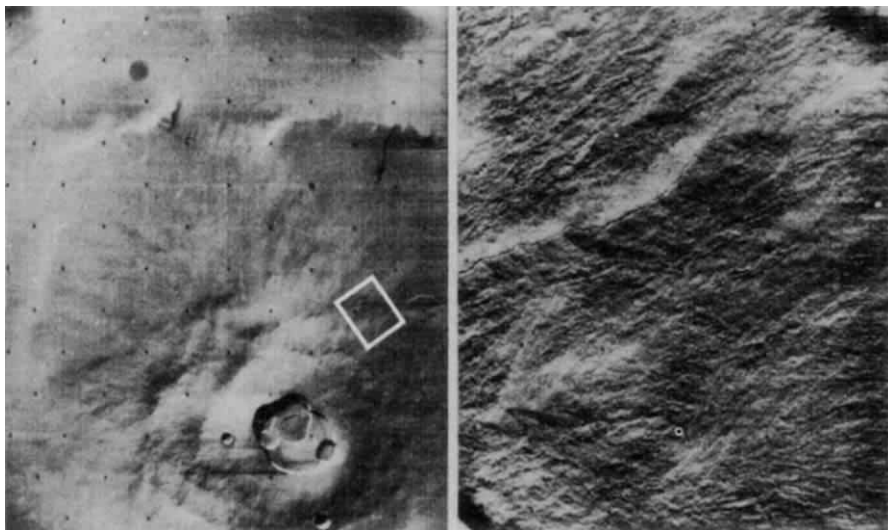
When Mariner 9 arrived at Mars on November 13, 1971, the television cameras revealed a nearly featureless disk. An enormous global dust storm was in progress. The experimenters

were able to observe the decline of this dust storm and then to observe, through a relatively clear atmosphere, almost the entire Martian surface to 1 km resolution. A proportion of the surface was also observed at about 100 m resolution.

Among the unexpected television findings reported by Mr H. Masursky (US Geological Survey) were the presence of large summit calderas at the peaks of immense volcanic piles, comparable with or larger than the island of Hawaii, the largest volcanic caldera on Earth. Surrounding the high caldera uplift area is an array of grabens, horsts and a rift valley system extending for 80° of Martian longitude and comparable in extent to the East African rift system. Most of these features are relatively uneroded and indicate significant tectonic activity on Mars in geologically recent times—a conclusion few planetary scientists had expected before Mariner 9.

Equally startling are a number of meandering dendritic rilles, which do not exhibit craters at the sources of their tributaries. They are unlike collapsed lava tubes and Hadley Rille in the lunar Apennines and are difficult to understand without invoking liquid water on Mars, a heretical conclusion; the low pressures on Mars (mean values 5 to 7 millibars) are on the wrong side of the triple point for liquid water to exist. The source of the water might be the localized melting of subsurface permafrost or, in a view proposed by Sagan before the Mariner 9 mission (*Icarus*, 15, 511; 1971), due to a periodic climatic instability on Mars connected with precession of the equinoxes and the large reservoir of volatiles in the polar caps. Liquid water on Mars in comparatively recent times greatly enhances the likelihood of indigenous life on the planet, and underscores the continuing need for sterilization of Mars landing craft.

Ultraviolet spectrometer results, reported by Professor C. Barth (University of Colorado), give a Martian exosphere temperature of about 315 K, slightly less than reported by Barth's experiment aboard Mariners 6 and 7 in 1969. With the measured exospheric number density of atomic hydrogen, Barth calculates a Jeans escape rate of hydrogen of about $2 \times 10^8 \text{ cm}^{-2} \text{ s}^{-1}$, corresponding—if photodissociation of water is the source of the hydrogen—to the loss of several metres of liquid water over geological time. Alternatively this hydrogen may represent a steady state between escape and arrival from the proton flux in the solar wind. The oxygen resulting from water vapour photodissociation may escape from the planet by photoionization of O^+ , and attendant superthermal ion-electron recombination. Barth argued that O^+ is



Nested pictures taken by Mariner 9 of the Nix Olympica region of Mars. The surface details shown in the telephoto picture—feathery texture, elongated lobes suggesting flow of material and a raised ridge with an irregularly cracked crest—are similar to those seen on terrestrial lava flows.

the major ion in the Martian ionosphere. Among neutral constituents CO_2 is the dominant component to 250 km, above which atomic oxygen dominates. Ozone has been found in the polar regions, and may be formed on CO_2 frost, although the polar caps probably have a mixed $\text{CO}_2/\text{H}_2\text{O}$ composition.

Professor C. Leovy (University of Washington) described the use of Mariner 9 as a Martian weather satellite. Many cases of detached hazes at the Martian limb and of lee wave clouds propagating from the high calderas are interpreted in terms of water ice clouds. The development of major frontal systems in the northern hemisphere has been uncovered. A principal problem is to understand how dust can be uniformly mixed to 35 km during the dust storm when the deposition of solar energy onto the dust produced an atmospheric temperature gradient significantly subadiabatic and therefore a stable atmosphere resisting vertical convection. Nevertheless, Professor Leovy believes that the heating of suspended dust in the tens of microns size range produces vertical velocities of a few metres per second, and enough turbulent support to explain the observations. He also presented a striking case of a bright dust cloud, moving over a Martian terrain in the course of a few days, and darkening the underlying surface, consistent with Sagan and Pollack's wind-blown dust theory of Martian albedo changes (*Nature*, 223, 791; 1969) in which dust storms remove fine bright particles and expose underlying darker material.

The search for variable features on Mars was described by Professor C. Sagan (Cornell University). A large number of cases have been found, in computer-processed photographs, of

albedo variations, or more dramatically, the appearance of new albedo features in a period of time ranging from days to weeks. An array of bright and dark tails, many of them emanating from craters, have been revealed, which can be understood in terms of the preferential deposition of bright material in depressions in the waning stages of the dust storm and the subsequent blowing of the dust out of the craters. Thus the tails, he said, may provide natural wind direction indicators and anemometers on the Martian surface. Cases of wind shadowing and of the apparent movement of dark material as well as bright material complicates the unravelling of aeolian stratigraphy on the planet.

The loci of bright and dark streaks in such areas as Syrtis Major conform rather well to the classical boundaries of these regions, and it is proposed that both the configuration of bright and dark areas on Mars and their seasonal and secular changes can be understood in terms of windblown dust. Because of the low atmosphere density wind velocities in excess of 70 m s^{-1} above the surface boundary layer appear necessary to initiate particle motion on the surface.

WATER

Pollution and Disease

from a Correspondent

MODERATION might have been the watchword of many of the participants who gathered in Washington DC on April 17 and 18, under the auspices of the Water Pollution Quality Research Council. The sixth international water quality symposium covered many aspects of water pollution and disease,

but the proceedings began with a verbal battle, billed as a dialogue between Professor Barry Commoner (University of Washington, St Louis) and Mr J. E. Kinney, a sanitary engineering consultant from Ann Arbor. The topic on the agenda was "Can Man Survive?" Professor Commoner predicted that man will survive in spite of his many environmental problems. Mr Kinney chose to concentrate on criticisms of his opponent's recent book *The Closing Circle*. The ensuing exchange greatly enlivened the audience, and Mr Kinney's approach set the mood for much of the symposium.

Discussing the association between heart disease and water hardness, Professor T. W. Anderson (University of Toronto), an epidemiologist, said that he is 90 per cent sure that there is a positive correlation. Several studies have indicated that there is a lower death rate from heart disease in regions where the water is hard, and Professor Anderson thinks that there is a substance *X* responsible for the phenomenon. Substance *X* might be a detrimental factor in soft water, and cadmium and sodium have been suggested. Professor Anderson dismissed sodium as a very unlikely candidate for substance *X*. On the other hand, *X* might be a beneficial factor in hard water, and he feels that calcium and magnesium are possible candidates. He suspects that substance *X*, whatever it may be, has an indirect effect on heart muscle, and does not influence the coronary arteries.

Soft water emerges with greater glory, however, from a study by another epidemiologist, Professor G. W. Comstock (Johns Hopkins University School of Hygiene and Public Health). He has shown a lower death rate from heart disease for white males, aged 45-64, if they drink soft water. This study also revealed that the risk of fatal arteriosclerotic heart disease was twice as great for people attending church infrequently than for those who attended once a week or more. Professor Comstock was modestly sceptical that his work proved anything except that because so many variables are involved it is almost impossible to do meaningful research on this subject.

One safe conclusion seems to be that there is no reason yet to recommend that soft water supplies be hardened to prevent heart disease, although it might one day seem necessary to manipulate the trace elements in drinking water in the interests of public health. The possibility that carcinogens may be found in water supplies was discussed by Dr M. R. Zavon (Huntingdon Research Center, Towson, Maryland). He cited radium as a carcinogen that is found in some well water and is removed by ion exchange water softening pro-

cesses. Dr Zavon cautioned that the chemist's ability to detect and identify trace substances in the environment has surpassed the ability to interpret the significance of his findings.

Would-be pollution controllers were sharply criticized by several speakers. There was special derision for the proposal that the discharge of pollutants into navigable waters in the United States should be eliminated by 1985, as set down in the Federal Water Pollution Control Act now before Congress. Dr R. Kunin (Rohm and Haas Co.) said that such a goal is unrealistic and fails to protect the interests of the public. He cited several elements, including boron, manganese, iron, copper and cobalt, which are necessary for health in certain concentrations, although toxic in greater quantities. Mr E. F. Johnson (American Water Works Association) condemned extremism in water pollution control for diverting the attention of both the public and the scientific community from important questions concerned with water supply. He said that priorities have been distorted to the point where the fish kills of the present have taken on greater significance than the possible people kills of tomorrow.

ENZYMES

Loose Connexions

from our Molecular Biology Correspondent

THE physical nature of the coupling between interacting sites in enzymes remains one of the more absorbing pre-occupations within the trade. One approach is to look for anomalous variants, in which the coupling is defective, and an interesting example that has now been described is an aberrant form of the celebrated enzyme aspartate transcarbamylase. One can now state, without inviting opprobrium, that the native molecule contains twelve chains, six each of regulatory and catalytic, the former disposed in pairs and the latter in groups of three. Two kinds of interaction prevail in aspartate transcarbamylase: cooperativity between catalytic sites and suppression of the catalytic activity by the binding of feedback inhibitors on the regulatory subunits. There is a mutant of *Escherichia coli* requiring uracil, which when derepressed with thiouracil, synthesizes a form of aspartate transcarbamylase that no longer displays interaction of catalytic sites. The nature of this variant has now been examined

Biochemistry of DNA Replication

Nature New Biology next Wednesday (May 17) publishes two reports germane to an unsolved question, which, ever since Watson and Crick discovered the structure of DNA, has tantalized molecular biologists; namely, the question of how DNA is semi-conservatively replicated *in vivo*.

To date three DNA polymerases (I, II and III) which use as substrates the deoxynucleotide triphosphates have been identified in *Escherichia coli*. DNA polymerase I, the Kornberg enzyme, is not apparently essential for the replication of the *E. coli* chromosome, but the properties of *dnaE* gene temperature sensitive mutants suggests that DNA polymerase III may very well be essential for this process. It might therefore have been predicted that the replication of the genomes of all *E. coli* plasmids would also depend on the presence of active DNA polymerase III and be independent of DNA polymerase I activity. Goebel's investigation of the replication of the DNA of the plasmid colicinogenic factor *E₁* in *E. coli*, however, completely confounds any such expectations.

In brief Goebel has found that although several plasmids with large genomes fail to replicate in mutant *E. coli* lacking DNA polymerase III but will replicate in cells lacking DNA polymerase I as expected, colicinogenic factor *E₁*, which has a very small genome, behaves in precisely the reverse

manner. Its DNA replicates quite happily in non-dividing *E. coli*, which lack DNA polymerase III, but fails to replicate in dividing *E. coli* which lack DNA polymerase I. These observations, together with Kingsbury and Helinski's observation that *E. coli* mutants lacking DNA polymerase I fail to maintain this plasmid, indicate that DNA polymerase I is essential for the replication of colicinogenic factor *E₁* DNA. And that implies, as Goebel says, "that more than one basic mechanism of DNA replication may be operating in *E. coli*".

Also in next Wednesday's *Nature New Biology* Brown *et al.* describe their attempts to discover how 6-(*p*-hydroxyphenylazo)-uracil (HPUra) blocks DNA synthesis in *Bacillus subtilis* cells treated with toluene to render them permeable to nucleotides. The chief conclusions of their work are, first, that the DNA synthesis in such permeabilized cells is probably true semi-conservative DNA replication and can be as sensitive to HPUra as DNA replication in intact cells and, second, that it is probably not HPUra itself that blocks replication but a metabolite obtained by biochemical reduction of the drug.

But in any event what Brown *et al.* have to say draws attention to the usefulness of HPUra and toluenized *B. subtilis* cells as an experimental system for probing DNA replication.

by Kerbiriou and Hervé (*J. Mol. Biol.*, **64**, 379; 1972).

It emerges in the first place that the mutant enzyme is not simply devoid of regulatory subunits, for electrophoretically it differs only slightly from the normal form, and dissociates under denaturing conditions to give the full complement of catalytic and regulatory subunits, both with normal molecular weight. In terms of pH-dependence and lability to heat, the rogue enzyme behaves normally, but when the initial reaction rate is measured as a function of substrate concentration there is no characteristic sigmoidal profile. The feedback inhibitor, CTP, does, however, repress the activity, though to a somewhat smaller degree than in the wild type. Kerbiriou and Hervé have established that thiouracil has no noticeable effect on the functional properties of normal aspartate transcarbamylase, and there seems to be no doubt that its effect is indeed to provoke an anomaly in the biosynthesis of the protein, possibly by contriving to introduce itself into the messenger. The physical basis for the uncoupling of the two kinds of interaction is an open question, though it has in the past been reported to be achievable by artificial means—either by introducing ATP into the system or by working at the alkaline extreme of activity. The enzyme in any case is presumably incapable of escaping from its high-affinity state.

A simpler example of cooperativity, involving only interactions between identical sites, is provided by yeast glyceraldehyde 3-phosphate dehydrogenase, for which Kirschner, in something of a landmark of recent enzymology, demonstrated the existence of a true concerted interaction mechanism. An interesting property of this enzyme is its strikingly different binding behaviour towards its natural cofactor, NAD, compared with NADH, which is the product of the physiological reaction. This has now been clarified in a dauntingly thorough study by von Ellenrieder, Kirschner and Schuster (*Europ. J. Biochem.*, **26**, 220; 1972) and the results give rise to some intriguing implications for the cooperative mechanism.

Kirschner's earlier kinetic experiments were all done at 40° C, where the sigmoidal character of the NAD saturation curves is strongly in evidence. It now transpires that at 20° C there is also cooperativity when one looks hard enough. From the temperature-jump kinetics, the forward and reverse rate constants for the conformational transition between the low- and high-affinity states of the enzyme can be extracted, and lead to an estimate for the position of the equilibrium between these states in the absence of cofactor.

An independent method of measuring

this equilibrium directly was also devised: the rate of reaction of a reactive thiol, which is present at each active site, with a chromophoric reagent is a characteristic of the conformational state. The admixture of faster and slower reaction rates measures the relative concentrations of the two. The two methods agree in the result that 90 per cent of the enzyme is in the low-affinity state under the given conditions. These affinities, of course, are defined in terms of NAD; the binding of NADH, on the other hand, is both weaker and hyperbolic, and the relaxation process corresponding to the conformational transition fails to manifest itself. Evidently, therefore, the high- and low-affinity states have indistinguishable affinities when it comes to binding NADH. This is further borne out by kinetics of displacement of NADH by NAD, which show that, irrespective of the presence or absence of the former, the conformational step is unaffected. The kinetics of the thiol reaction in the presence of ligand also indicate that NADH has no preference for either state over the other. A competitive reaction between NAD and NADH, which controls the level of activity of the enzyme may, the authors suggest, be important in the cell.

The most interesting inference from these results, however, arises from the

finding that the low-affinity state has essentially the same binding constant for the two ligands, whereas, of course, these binding constants in the high-affinity state differ vastly. It has been suggested for this enzyme, and found by Rossmann and his colleagues for a different dehydrogenase (lactate), which also has NAD as cofactor, that there are two operationally separate binding sites for the adenine and nicotinamide parts of the ligand. The suggestion, then, is that as between the two states, the adenine site remains undisturbed, and in the low-affinity state binds NAD and NADH unassisted. In the high-affinity state the second site is no longer dormant, and manifests a large preference for a pyridinium over a dihydropyridine ring.

Subsites for different parts of a ligand are in general no new idea, and in simple hydrolytic enzymes many cases have been documented in which a secondary substrate specificity is controlled by the subsites. Berger devised an interesting strategy of measuring activities of proteases on pairs of peptides in which individual residues at different distances from the point of cleavage are replaced by their antipodes. If there is interaction with the enzyme at this point in the chain, the hydrolysis rate will be affected.

Harper and Berger (*Biochem. Bio-*

Transcriptional Genetic Mapping

THE identification of sets of genes in bacteria which are expressed in a co-ordinated fashion, in other words the identification of operons and the determination of the order of the individual genes in an operon, has been achieved by sophisticated genetic mapping experiments. The simple genomes of many bacteria and phages are amenable to such investigation, but the genomes of eukaryotic cells are too large and at present too poorly characterized to be analysed by high resolution genetics. The experiments reported by Webb and Bleyman in *Nature New Biology* next Wednesday (May 17), however, at least raise hopes that it may be possible to identify operons in eukaryotic cells, if indeed they exist there, by transcriptional mapping, a technique which was first described by Bleyman and Woese.

Actinomycin D binds to DNA and introduces at random blocks to the transcription enzyme RNA polymerase and this process can be described by a target theory model. To illustrate the possible use of this process and model for genetic mapping, Webb and Bleyman analysed the pattern of expression of the *lac* operon of *Escherichia coli* in cells exposed to EDTA to render them permeable to the antibiotic; the EDTA treatment alone has no detect-

able effect on transcription. The first cistron of the *lac* operon, which specifies β galactosidase, is four times the size of the third and last cistron which specifies transacetylase. This means, of course, that the β galactosidase cistron has a target size four times that of the transacetylase cistron. But any actinomycin D block in either the first or the second cistron will prevent the transcription of the last, transacetylase cistron because the *lac* operon cistrons are transcribed into a single messenger RNA.

Target theory predicts therefore that although the β galactosidase cistron is bigger than the transacetylase cistron the expression of the second will be more sensitive to actinomycin D than the expression of the first, and a simple calculation indicates the difference in sensitivity will be about 1.5-fold. When Webb and Bleyman measured the amount of the two enzymes made in cultures exposed to various amounts of actinomycin D they found, gratifyingly, that production of transacetylase was 1.6 times more sensitive to inhibition than the production of β galactosidase, a result which indicates that transcriptional mapping is not only collinear but also cometric with classical genetic mapping.

phys. Res. Commun., 46, 1956; 1972) have now applied this method to pronase, and find that the extent of the active site is such that it interacts with five successive residues on the substrate. Beyond this there is no measurable effect of sequence. In pepsin, Sachdev, Johnston and Fruton (*Biochemistry*, 11, 1080; 1972) have also shown that the affinity for substrate peptides depends acutely on the side chains near the vulnerable bond. This will presumably turn out to be a very general phenomenon.

MYRIAPODOLOGY

Myriapods in Manchester

from a Correspondent

In his presidential address to the second international congress of myriapodology at the University of Manchester (April 5-12), Dr S. Graham Brade-Birks (Wye College, Kent) looked back over fifty years to the foundations laid by Brölemann, Verhoeff and Pocock, and forwards to an increasing awareness of the importance of myriapods in soil science. Delving still further back into one of the president's earlier fields of research, Professor O. Kraus (University of Hamburg) considered that Palaeozoic diplopods may have lived above ground, only later going to earth as cryptozoa, loosing their elaborate spines and fusing their sclerites into rigid cylinders. These "rings", according to Dr W. Dohle (Free University, Berlin), have a complex embryological origin. The first diplosegment (the fifth ring) encompasses the single pair of limbs of the fourth and the anterior pair of the first ventral diplosomite. Such an explanation, apparently highly improbable, he said, is, however, true. More highly improbable schemes of the diplopod nature of "segments" in other groups were challenged by Dr S. M. Manton (British Museum (Natural History) and Queen Mary College, London) who furnished explanations of the extra tergites in Symphyla, and other departures from simple segmentation in chilopods, in functional terms, as devices facilitating turning, running or burrowing.

In a free discussion on phylogeny several schemes were examined. The scheme of Tiegs, which relates Pauro-poda and Diplopoda as Dignatha and Chilopoda, Symphyla and Hexapoda as Trignatha found most favour, but Dr Manton argued emphatically against relating any myriapod with a hexapod group and urged caution in relating any two myriapod groups. Myriapods may be loosely connected by their jointed transversely biting mandibles, but are quite distinct from the insects with unjointed rolling mandibles.

Interrelations between species were also debated. Dr E. H. Eason (Moreton-in-Marsh, Gloucestershire) described the confusion which had arisen from the "lumping" of Attems and the "splitting" of Chamberlin and the odd zoogeography of the Lithobiidae which might follow. Dr C. A. W. Jeekel (Zoological Museum, University of Amsterdam) has revised the Sphaerotheriidae (giant pill millipedes) using secondary sexual characters, and has produced natural groupings more consistent with their discontinuous geographic distribution. One of the highlights of the congress was a recording of a stridulating male *Sphaerotherium* made by Dr U. Haacker (University of Hamburg) as part of his study of communication between potential mating pairs of millipedes. Dr Haacker showed the functional significance of many of the secondary sexual characters used by systematists.

Excellent contributions on the neurosecretory organs of diplopods were presented by French physiologists who have been largely responsible for opening-up this area of research. Professor F. Sahli (University of Dijon) reviewed his own work and that of his French and Indian colleagues and moved towards a synthesis. The cerebral glands (organes de Gabe) seem to be the histophysiological equivalents of the neurohaemal organs of the apterygote groups and the Symphyla; he compared the paraoesophageal bodies with the corpora cardiaca of pterygotes and tentatively suggested that the corps connectifs are analogues of the perisymphathetic bodies of pterygotes.

Studies on post-embryonic development have been facilitated by the methods of stadium characterization developed by French workers. Mr J.-M. Demange (Muséum National d'Histoire Naturelle, Paris) presented some interesting thoughts on the increment of segments and ocelli added at

each moult and reaffirmed his belief in Brölemann's theory of neotenic contraction. One of the practical results of this work is that in Manchester life tables have been constructed for several species which, along with feeding studies, confirm that a large part of the leaf fall in woodland is ingested and comminuted by millipedes. It is still difficult, however, to characterize the epimorphic stadia of pill millipedes, but this problem has been surmounted by Mr K. L. Bocock and his colleagues (Nature Conservancy, Merlewood Research Station, Lancashire). Most exasperating are the difficulties encountered in ageing blaniulids (thin snake millipedes), some of which are important pests of sugar beet.

More needs to be known about the ecology of these animals if sensible control measures are to be developed. Woodland millipedes are apparently more favoured subjects and there were three complementary contributions on *Proteroiulus fuscus* in woodland, one by an English and two by Finnish zoologists. International cooperation fostered by the Centre National de Myriapodologie in Paris assured the success of this symposium, the first to enjoy the sponsorship of the Zoological Society of London away from the capital.

AGRICULTURE

Seed Ecology

from a Correspondent

THE nineteenth annual Easter school in agricultural science, held at the University of Nottingham School of Agriculture at Sutton Bonington from April 17-20, had for its subject the wide-ranging topic of seed ecology. During the meeting, this topic was considered at all levels, ranging from the fundamental biochemistry of dormancy and

Arrangement of Ribosomal Proteins

In next Wednesday's *Nature New Biology* (May 17) Kagawa, Jishuken and Tokimatsu describe an attempt to determine how the ribosomal proteins of the *Escherichia coli* 50S subunits are arranged along the RNA chain. When the ribosomes are deprived of magnesium ions, they unfold to give extended, nuclease-labile particles. Kagawa *et al.* have digested these particles with ribonucleases T₁ and I, and separated the nucleoprotein fragments so produced by polyacrylamide gel electrophoresis.

The components in the mixture were then subjected to electrophoresis in the second dimension under solvent conditions leading to dissociation of the pro-

teins from the RNA and each other. By this means the proteins present in particular fragments were identified. By using different concentrations of EDTA to bring about progressive unfolding, the extent of digestion can be controlled. Based on the results, the authors present a linear sequence of proteins, which is the presumed order in which they occur.

Not all components were resolved: the authors allocate twenty-six resolved 50S ribosomal proteins to twelve positions along the RNA. No allowance is made for proteins binding to other proteins and not to RNA, and it has to be assumed that the unfolding process is not accompanied by any redistribution of proteins.

germination, to agricultural aspects of the establishment and protection of seedlings.

In a discussion of the hormonal relationships of dormant seeds, Professor P. F. Wareing (University College of Wales, Aberystwyth) showed that during the chilling treatment of dormant sycamore seeds, a marked but transient increase in levels of endogenous cytokinin occurs. Similarly, in the light-requiring seeds of *Rumex obtusifolius*, a very rapid far-red reversible increase in cytokinin levels occurs upon exposure of the seeds to red light. These results were taken to indicate that the role of endogenous cytokinins in seed dormancy, previously largely ignored, should be investigated in more detail.

In a further contribution from Aberystwyth, Dr M. A. Hall presented evidence that red light, acting through phytochrome, interacts with ethylene in controlling the germination of several weed species and it was suggested that such a response might be ecologically important in compacted or waterlogged soils with high levels of ethylene. Still at the hormonal level, Dr Y. Guterman (Hebrew University, Jerusalem) showed that the germination behaviour and seedling growth of *Lactuca scariola* can be influenced by treatment of the parent plant with growth regulators. Furthermore, in other species tested (for example, *Ononis sicula*) the photoperiodic conditions under which seed maturation occurred on the mother plant were shown to affect germination behaviour.

A series of contributions by Dr D. Côme (Plant Physiology Laboratory, Meudon), Dr M. M. Edwards (University of Nottingham) and Professor E. H. Roberts (University of Reading), on the uptake and subsequent metabolism of oxygen by dormant seeds, provoked a lively controversy concerning the role of the seed coat. The consensus of opinion seemed to be that although the coats restrict oxygen uptake and thus reduce respiration rates, this effect is not responsible for the dormancy of the enclosed embryo. Considerable interest was centred on the metabolic and ultrastructural changes occurring during prolonged storage of seed. Dr B. Roberts and Dr D. Osborne (ARC Unit of Developmental Botany, Cambridge) presented evidence that the major lesion in non-viable rye embryos could be traced to the transferase I factor in isolated protein synthesizing systems. Subsequently, Dr N. Hallam (Monash University) showed an intriguing series of electron micrographs demonstrating disruption of cellular structure (particularly of mitochondria) in non-viable seeds. Some seeds in this study were more than 6,000 years old and had been obtained from ancient Egyptian grain silos.

In a discussion of seed storage prob-

lems Professor T. A. Villiers (University of Natal) showed how air-dried seeds are much more susceptible to storage damage than fully imbibed, dormant seeds. He attributed this, on the basis of ultrastructural observations, to the inability of the natural repair mechanisms to operate in air-dried seeds, thus allowing normal membrane damage consequent upon storage to be expressed in the form of decreased viability.

In a session devoted to variation in seed germination behaviour, Dr W. J. Whittington (University of Nottingham) demonstrated by statistical techniques that both genetic and environmental factors influence seed variability with respect to dormancy and to speed of germination. Furthermore, it could be shown that genetic factors are more important in wild than in cultivated populations. Dr P. A. Thompson (Royal Botanic Gardens, Kew) discussed the geographical adaptation of seeds, especially with regard to effects of temperature on germination, and showed how these responses could, for many wild species, be correlated with what is known, from other evidence, of their geographical origin. It was also suggested that many cultivated plants, including lettuce, leek and several forms of *Brassica*, have maintained throughout cultivation temperature responses appropriate to the geographical distribution of their wild progenitors.

Summing up, Dr I. H. Rorison (University of Sheffield) stressed the necessity for plant physiologists and biochemists to recognize the ecological significance of their investigations and to select species with relevant natural distribution patterns. The importance of seed ecology to agricultural production and to the maintenance of natural ecosystems is such that greater cooperation and cross-fertilization between plant scientists of varying disciplines, as exemplified by this meeting, is needed.

TUMOUR VIROLOGY

Searching for Proteins

from our Cell Biology Correspondent

SEARCHING for the proteins specified by a small viral genome, such as the 3×10^6 daltons of SV40 or polyoma virus DNA which can code for only about 200,000 daltons of polypeptide, in a mammalian host cell that continues to metabolize after infection really demands the sort of optimism and perseverance shown by the proverbial searcher for the needle in the haystack. But if the rewards of success promise to be great enough the job will be tackled sooner or later. Walter, Roblin and Dulbecco (*Proc. US Nat. Acad. Sci.*, **69**, 921; 1972), for example, have of late been comparing by SDS polyacrylamide gel electrophoresis the poly-

Histones and Chromatin Structure

DURING the past several years much has been discovered about the chemistry of the histone proteins of eukaryotic cell nuclei and one outstanding fact has emerged. It is now abundantly clear that the amino-acid sequences of these proteins have, during the course of evolution, remained extraordinarily stable. That fact alone, as Johns points out in next Wednesday's *Nature New Biology* (May 17), indicates that histones probably have some important structural function to do with the packing of DNA for which the structure of the whole histone molecule is crucial.

Indeed Johns contends, in part from the results of a series of experiments in which he measured the capacity of the C-terminal and N-terminal halves of F2B histone to precipitate DNA and to block its activity as a template for RNA synthesis, the mere binding of histones to DNA does not inevitably result in the restriction of that DNA's capacity to act as a template for transcription. Rather he suggests that histones are structural proteins part of which bind strongly to DNA and part of which are free to interact with other nuclear macromolecules. The physical state of any chromatin will determine whether or not it is an efficient template for RNA

synthesis and the physical state of the chromatin will depend on precisely which molecules interact with the "free" part of histone molecules.

Pursuing a rather similar vein, Sutton, in the same issue of *Nature New Biology*, proposes a model of the structure of chromatin and gene control based on the idea that regions of chromatin with very similar or identical chemical composition will have a similar three-dimensional structure and as a result tend to "crystallize" or pack themselves in a stable and regular array. He suggests that the large tracts of DNA with repetitive base sequences, which may account for as much as 80-90 per cent of the total DNA, are likely to "crystallize" into heterochromatin.

Obviously the strength of the packing forces holding such "crystals" together will depend on the accuracy and extent to which particular sequences are repeated and the conformation the DNA histone complex adopts. Once "crystallized" the DNA would be unavailable as a template for transcription and so this process can be envisaged as providing a coarse control of gene expression on which may be superimposed specific fine control mechanisms perhaps similar to those operating in bacteria.

peptides of SV40 particles with those in uninfected monkey cells and monkey cells infected by this virus, in an apparently successful attempt to identify polypeptides which may well be specified by SV40.

Walter *et al.*, like most other groups who have electrophoresed on gels the polypeptides liberated when SV40 particles are disrupted, find that two polypeptides with molecular weights of about 44,000 and 31,000 account for about 83 per cent of the total polypeptide of the virions. In addition they find as many as twelve other minor polypeptides in preparations of disrupted virions, at least some of which may well be coded by the host cell and others may be degradation products.

Of the protein made in cells after infection, about 10 per cent proves on electrophoresis to be identical to the two major polypeptides of the virus particles. Almost all of the other proteins made in infected cells can also be found in uninfected cells and must presumably be specified by the cell genome. But about 0.5 per cent of the protein which has accumulated at late times after infection can be resolved on electrophoresis into three polypeptides, the molecular weights of which can be estimated to be 70,000, 60,000 and 8,000.

These three polypeptides are not found in extracts of uninfected cells, neither are they components of the SV40 particles and, therefore, adopting the cumbersome but familiar terminology devised by investigators of polio virus replication, Walter *et al.* designate them respectively as NVP1, NVP2 and NVP3. The first two of these three polypeptides are apparently accumulated in the nuclei of infected cells, and polypeptides of similar molecular weights can also be detected in Balb/3T3 cells infected with polyoma virus.

Walter *et al.* believe that the two major virion proteins, which can readily be detected in infected cells, are primary gene products, for the kinetics of their labelling and their half-lives argue against the idea that these two molecules are derived by cleavage maturation from a larger precursor molecule. Furthermore, because they comprise 10 per cent of the protein made in infected cells they are probably specified by the viral genome. The three non-virion proteins—NVP1, NVP2 and NVP3—also might be coded by the virus because they are restricted to infected cells. If that is so, these polypeptides, together with the two virion proteins, account for the total coding capacity of the viral DNA.

As Walter *et al.* cryptically remark, the facts that studies with temperature sensitive mutants of polyoma virus indicate that they are two and possibly three early genes, and that SV40 infected cells acquire three new antigens (T anti-

gen, U antigen and transplantation antigen), may not "represent a fortuitous agreement" with their detection of three non-virion polypeptides in infected cells. Of course, if these three polypeptides really are specified by the viral genome one or more of them must presumably be involved in maintaining the transformed cell phenotype of non-permissive cells transformed by SV40 and the identification of the "transforming" gene product is the ultimate goal of this work.

It would be wrong, however, to create the impression that the hunt is about to make a kill, because the alternative possibility, namely that one, two or all of the non-virion polypeptides are coded by cell genes, the expression of which is specifically induced by SV40 infection, has not in any rigorous way been excluded by the data obtained to date.

INSULIN

Binding Process

from a Correspondent

THE interaction of insulin with membranes is now attracting a great deal of interest, particularly because of recent work on the nature of the interaction of the hormones of insulin with liver plasma membranes and with membranes from isolated fat cells. This work is also taking place at a time when the three-dimensional structure of insulin has been elucidated so that there is increasing speculation about the nature of the residues on the surface of the insulin molecule which may be important in the binding process.

Set against this background the recent report by Gavin, Roth, Gen and Frey-

chet (*Proc. US Nat. Acad. Sci.*, **69**, 747; 1972) concerning insulin receptors that have been identified on human lymphocytes is particularly interesting. These authors have shown that insulin and some of its derivatives can bind to human lymphocytes. The experiments have been carried out by measuring the displacement of insulin labelled with iodine-125, bound to lymphocyte membranes by insulins of various species and insulin derivatives (such as insulin lacking the C-terminal alanine or the 8C-terminal acids of the B chains). The manner in which these substances bind to lymphocytes very closely resembles the way in which they stimulate glucose oxidation in adipose tissue cells, and the results suggest important analogies between binding sites for insulin on human lymphocytes, on fat cells and on liver cells with regard to the kinetic properties and temperature dependence of the binding reaction.

Whether insulin has an important physiological role in lymphocyte metabolism is still largely open to question, but a recent report by Haddep and his colleagues (*Nature New Biology*, **235**, 174; 1971) suggests that insulin may at least affect a lymphocyte plasma membrane ATPase. It may be that even if lymphocytes are not a very usual target tissue for insulin, typical receptor sites concerned with the binding of this hormone to cells are nevertheless present. Because lymphocytes are relatively easily obtained from human blood they may, as Gavin *et al.* suggest, be an ideal cell system in which to study insulin binding in man. Clearly work of this kind may have important implications for the further study of metabolic diseases such as diabetes, and it ought to be greatly extended.

Large Japanese Air Shower Examined

THE data on the very large extensive air shower which was reported last year by Suga *et al.* of the Institute for Nuclear Study, University of Tokyo (*Phys. Rev. Lett.*, **27**, 1604; 1971), are subjected to a critical analysis in next week's *Nature Physical Science* by Garmston and Watson of the University of Leeds. Suga and his colleagues claimed that the shower contained about 2×10^{12} particles at sea level—where it was detected with an array of scintillator detectors and spark chambers. The Japanese group suggested, on this basis, that the energy of the primary cosmic ray responsible for initiating the shower at the top of the atmosphere was about 4×10^{21} eV, the largest ever recorded.

Garmston and Watson take issue with this conclusion and propose that the data presented by the Japanese group last year are indicative of a primary energy of between 4×10^{19} eV and 1.4×10^{20} eV,

a factor of between 10 and 100 less than claimed. They say, for example, that depending on the shape assumed for the integral spectrum of the primary cosmic rays, only between 2×10^{-3} and 4×10^{-5} showers with an energy as high as 4×10^{21} eV would be expected during the running time of the Tokyo air shower array (3 years), taking into account the area covered by the detectors.

One of the difficulties in analysing the Tokyo air shower is that the core of the shower fell outside the area enclosed by the detectors, and it is therefore difficult to estimate the size of the shower and hence its energy. Garmston and Watson also examine very closely the energy spectrum which the Tokyo group assumes for the muons in the air shower and the way in which the density of air shower particles varies across the array itself.

Revolution in Liquid Chromatography

J. N. DONE, G. J. KENNEDY & J. H. KNOX

Department of Chemistry, University of Edinburgh

Liquid chromatography can now be performed in minutes rather than hours or days with a sensitivity and ease of operation previously associated only with gas chromatography. The new techniques promise to be of immense importance for the development of chemistry, biochemistry and medicine.

LIQUID chromatography (LC), the original form of chromatography devised by Tswett¹, did not gain widespread recognition until it was rediscovered by Lederer and co-workers in about 1930 (ref. 2). It was extended by Martin and Synge³ who invented liquid-liquid partition chromatography in 1941. This, in its turn, paved the way for paper chromatography⁴, thin-layer chromatography⁵ and gas chromatography^{6,7} (GC).

Gas chromatography was an immediate success because of the simplicity of the equipment and the wide range of its applicability. The technique and theory of the method developed rapidly, whereas, by contrast, the theory of liquid chromatography remained more or less static, at the stage reached by Martin and Synge³. Giddings^{8,9} was one of the first to recognize that the theory developed for gas chromatography was equally applicable with minor modifications to liquid chromatography. He showed that the performance which had previously satisfied liquid chromatographers could be greatly improved by paying attention to certain key features. Indeed, the nub of the problem in LC was the appreciation of the interrelation between pressure drop, particle size, eluent velocity, and column efficiency. The theory indicated that by using high pressures and very small particles LC could rival GC both in speed and resolving power. Although the value of using small particles had been appreciated by Hamilton in connexion with ion exchange chromatography¹⁰, and by Snyder for adsorption chromatography¹¹, their ideas were not generally assimilated and it was left to those who had received their principal training in gas chromatography to make the most notable advances. Particular credit must go to Kirkland of du Pont¹² who first showed how high speed separations could be achieved in a few minutes by using specially prepared layered particles about 30 μm in diameter and pressures up to 3,000 pound inch⁻². The emergent bands were detected by a high sensitivity ultra-violet photometer. Fig. 1 shows one of the first high speed liquid chromatograms published by Kirkland.

An important benefit of the application of the theory was a unification of the practice of LC. Modern equipment for HPLC is similar for all forms of liquid chromatography, and is equally usable for adsorption, partition and gel chromatography. Only for the chromatography of amino-acids on ion exchange resins is special equipment required, and this stems more from the special detection system and the slowness of the method than from any chromatographic peculiarities.

Equipment

Present equipment for HPLC (see, for example, ref. 13) mirrors many features familiar to gas chromatographers, and present trends are towards systems with the same versatility, ease of operation, cheapness and capacity for automation.

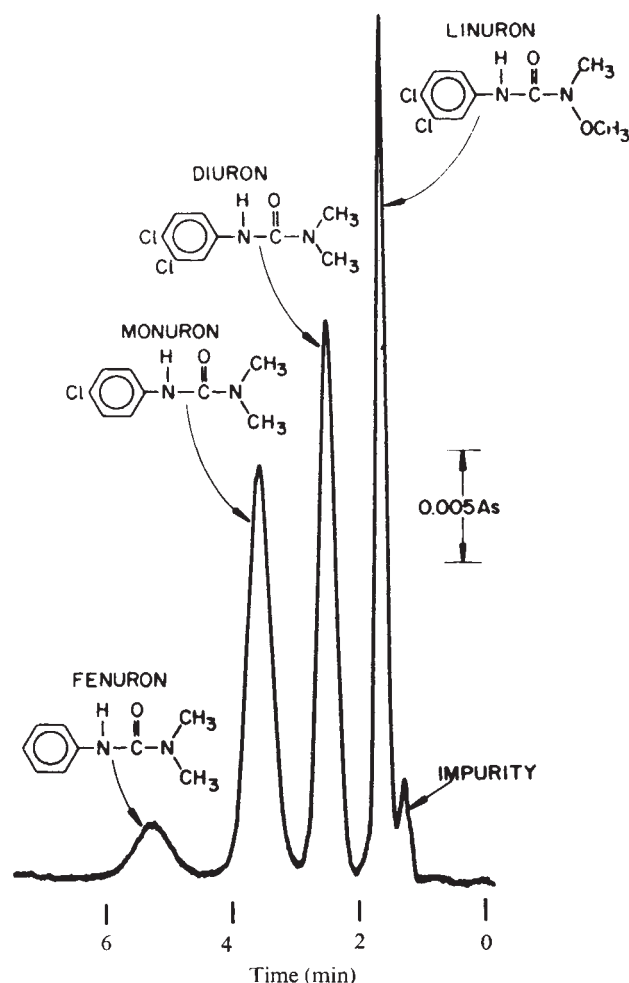


Fig. 1 Fast liquid chromatogram of urea herbicides, from ref. 12. Conditions: 1 m $\times$ 2 mm column packed with 40 μm 'Zipax' coated with 1% $\beta\beta'$ oxydipropionitrile (BOP); mobile phase, dibutyl ether; sample, 0.7 μg of each material; detector, ultraviolet photometer.

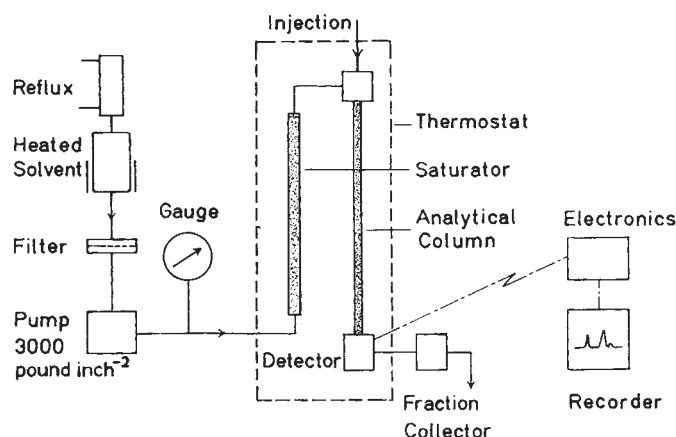


Fig. 2 Outline of HPLC equipment.

Fig. 2 outlines the essential parts. Degassed eluent is pumped at a pressure up to 3,000 pound inch⁻² through a column containing a finely divided and well graded partitioning material in the size range 10 to 50 μm . To handle the high inlet pressures, columns and fittings are usually of stainless steel, and packing materials are strong and rigid. Most are based on glass or silica. Columns are about 2 mm in diameter and 50 to 100 cm long. Flow rates are about 20 mm s⁻¹ so that unretained materials are eluted in about 1 min as is the practice in gas chromatography. When the partitioning agent is slightly soluble in the mobile phase, as in liquid-liquid partition chromatography, a pre-column or saturator must be placed before the analytical column to prevent stripping of the stationary phase.

Samples may be injected either by an injection valve or more often by a microsyringe: a typical injection might be 1 to 10 μl of a 1% solution of an unknown mixture. Surprisingly, 5 μl syringes, widely used in gas chromatography, can readily withstand pressures of 5,000 pound inch⁻² without shattering. The chief limitation of syringe injection is not the syringe itself but the rubber septum through which the syringe needle is inserted. When the pressure exceeds about 1,500 pound inch⁻² the septum must be protected.

The most decisive feature of current technology in LC is the use of highly sensitive post-column detectors. Like GC detectors, those used in HPLC produce an electrical signal proportional to the concentration of the eluted solute. Because column volumes are small, detector volumes must also be small, typically 10 μl . The most popular detectors are the refractive index (RI) monitor¹⁴ and the ultraviolet absorption photometer¹⁵. The RI monitor compares the refractive indices of the eluent and the pure mobile phase. Because it monitors a bulk property it is responsive to all substances to some extent, but it has the inevitable drawback of being somewhat insensitive. The best RI monitors will discriminate to about 10^{-7} RI units, but because the change in RI from one pure substance to another is of the order of 0.1 the concentration sensitivity is about 1 in 10^6 . Ultraviolet detectors, on the other hand, are much more sensitive but are limited to substances which absorb (for example, aromatic and carbonyl compounds). The limit of detection of a substance with a molar extinction coefficient of about 10^4 is about 1 in 10^9 . As in GC, sensitivity has generally to be bought at the expense of generality but, of course, in many applications selectivity is highly desirable, and very high sensitivities may be expected in such cases.

There are many other principles on which detection may be based. Polarography¹⁶, for example, has been used successfully for the analysis of pesticides and is capable of a on a pellet of adsorbent, is somewhat less sensitive. In sensitivity of about 1 in 10^8 . The micro-adsorption detector¹⁷, which uses the heat released by adsorption of eluted solutes transport detectors¹⁸ column effluent is collected on a wire or

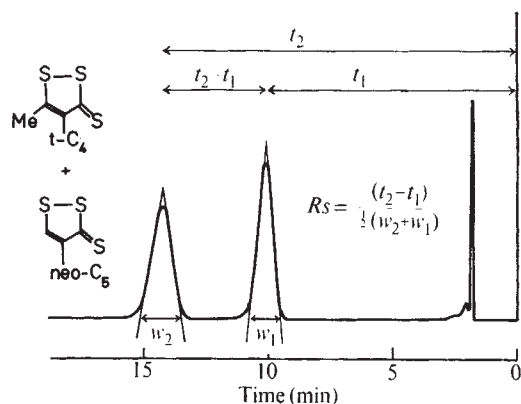


Fig. 3 Calculation of resolution. Separation of heterocyclic sulphur isomers on 'Corasil II' by adsorption chromatography; mobile phase, 1% CCl_4 in hexane.

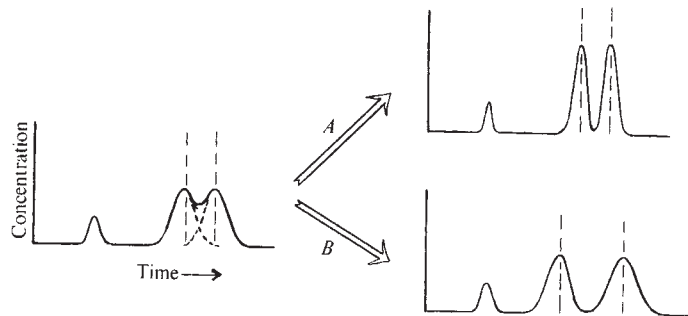


Fig. 4 Improvement of resolution. A, By making peaks narrower; B, by increasing peak separation. Initial peaks represent unretained solutes, and full vertical lines the moments of injection.

band which first passes through a low temperature oven where the solvent (necessarily volatile) is removed. Any involatile solute remains on the wire which next passes to a reactor where the solute is either pyrolysed or oxidized. The gaseous products from the reactor pass to a sensitive GC detector. Existing transport detectors are no more sensitive than RI monitors, but they can probably be greatly improved. They possess considerable versatility because they can be made specific for different elements including, of course, carbon.

Key to the Revolution

The achievement of high performance in chromatography requires an understanding of how resolution can be controlled and improved—for what every practising chromatographer wants is better resolution, in a shorter time, at a lower cost, and with less expense of effort.

Resolution in chromatography is defined as

$$\text{Resolution} = \frac{\text{Difference in times of peak maxima}}{\text{Mean peak width at base}} = \frac{(t_2 - t_1)}{\frac{1}{2}(w_1 + w_2)} \quad (1)$$

and the concept is illustrated in Fig. 3 by a separation of two sulphur heterocyclic isomers with a resolution of 2.8. A resolution of unity implies almost base-line separation. Incomplete resolution, a common problem, may be improved either by making the individual peaks narrower without changing their relative retention times, or by increasing the peak separation without changing their widths as shown in Fig. 4.

To increase separation without changing peak widths requires a change in the relative partition ratios of the substances between the mobile and stationary phases. This means changing the thermodynamic properties of the column. To obtain sharp peaks, chromatography must be carried out in very nearly perfect equilibrium conditions⁹,

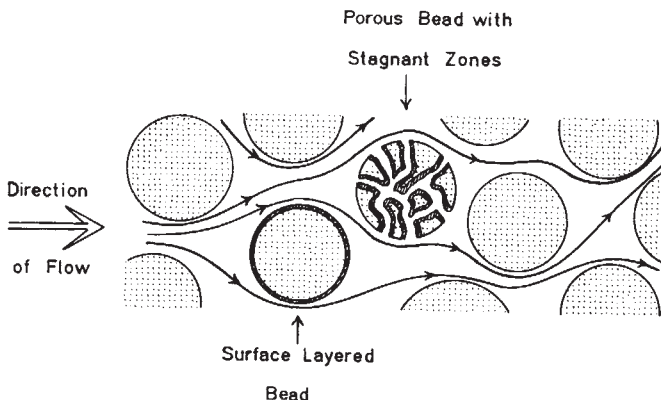


Fig. 5 Two dimensional representation of the flow regimes in a packed column, for porous and surface layered particles. —→, Flow lines; hatched borders, stationary phase; stippled areas, support structure.

and it is thus not possible to influence retention ratios by changing parameters such as the flow rate, particle size and column length. Changes in relative retentions can be obtained only by changing the stationary phase and/or the mobile phase. Although determination of the optimum combination of phases is important in any chromatographic study, the search for the ideal combination can be fruitless if the kinetic features of the equipment are poor, so that peaks are wide and elution times long. It is therefore vital to examine how resolution can be improved by making peaks narrower.

There are two sources of band spreading in any form of chromatography. The first is slow axial diffusion, and the second is the disturbance of equilibrium caused when one fluid flows over another fluid or over a solid. Only the second source of spreading is important in liquid chromatography as currently practised. In discussing flow-generated dispersion, three distinct regimes within any column may usefully be defined (Fig. 5); these are the stationary phase which is permanently held by the support (or may actually be the support), the stagnant part of the mobile phase which fills the interstices of the support, and the free flowing part of the mobile phase in the interparticle space. Within the flowing part, there is a whole spectrum of fluid velocities both across the column and along the column. The band spreading which velocity variations within and between these regions tend to produce can be countered in two ways; first, by molecular diffusion across the direction of flow, and second, by the tortuous nature of the flow itself, for no velocity, however extreme, is maintained for more than a few particle diameters downstream of any point in the column. The dispersion is generally reported in terms of quantity called the "height equivalent to a theoretical plate" or more simply the plate height (H) which can be thought of as the mean downstream equilibration distance within the column. Mathematical analysis of the column process allows H to be expressed in terms of column parameters such as the linear flow velocity, particle diameter, retention ratio, and diffusion coefficients.

When flow variations are countered only by lateral diffusion the equilibration time is constant, and therefore the equilibration distance measured downstream (that is, the plate height) is proportional to the fluid velocity, u . If, on the other hand, the dispersion is countered only by the tortuous nature of the flow itself, fluid velocity will have no effect on H , because the equilibration distance is governed by the geometry of the column. In practice both counter-dispersion mechanisms are important and the dependence of H on u is a compromise between $H=\text{constant}$, and $H \propto u$. Over about two orders of magnitude of u , H is found to follow a fractional power of u :

$$H = Cu^n \text{ where } 0 < n < 1 \quad (2)$$

Typical experimental dependences are shown in Fig. 6 for some column packings used in HPLC. n may be as low as 0.25 and as high as 0.7, but, generally speaking, a low exponent is desirable. At much lower velocities than those commonly used in HPLC, longitudinal diffusion becomes important. The dispersion so produced increases with the time of residence in the column, and, at very low velocities, H rises again as shown by the broken lines in Fig. 6.

The non-equilibrium theory of dispersion⁸ just described explains peak broadening in terms of a balance between the dispersive effects of flow variations across the column, and the counteracting effects of the tortuous downstream flow and transverse diffusion. As both the diffusion time and the "reticulation" of velocities over the column cross section

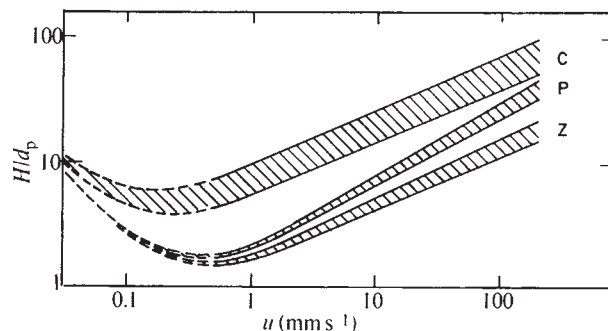


Fig. 6 Dependence of reduced plate height, H/d_p , on fluid velocity, u , for 'Zipax' (Z), 'Porasil' (P), and 'Chromosorb' (C). Lower limits of bands are for unretained solutes, upper limits for retained solutes giving maximum dispersion. Particle diameter $40 \mu\text{m}$.

depend on particle diameter, d_p , one predicts that better resolution will be obtained with smaller particles. This is well authenticated and is clearly shown by the chromatograms in Fig. 7. Columns were of identical size, and the elution time was kept constant for particle diameters between 22 and $115 \mu\text{m}$. One might well ask why even smaller particles (yielding still better resolution) have not been used. Apart from the unavailability of smaller particles, two problems arise when one tries to do this. First, smaller particles are more difficult to pack, and, because uniform packing is essential for high performance in chromatography, the expected improvement in performance does not always materialize if particular size is reduced too far.

Second, the driving pressure required for comparable elution speed increases as $1/d_p^2$, and the particle diameter does not have to be reduced much before the maximum pressure capability of most equipment is exceeded. If we assume that columns can be equally well packed whatever the particle size, it can be shown¹⁹ that the analysis times for the resolution shown in Fig. 7 with $22 \mu\text{m}$ particles can be greatly reduced if particle size and column length can be appropriately chosen (see Table 1). These figures are useful chiefly because they show how far we may still expect to improve the performance of existing equipment, and what technological problems are likely to be met during progress towards very fast liquid chromatography with elution times of seconds instead of minutes.

Although the theory suggests that the resolution shown in Fig. 7 for $22 \mu\text{m}$ particles could be obtained in only 4 s with a column 5 mm long containing $0.5 \mu\text{m}$ particles, the practical difficulties in achieving this would be insuperable,

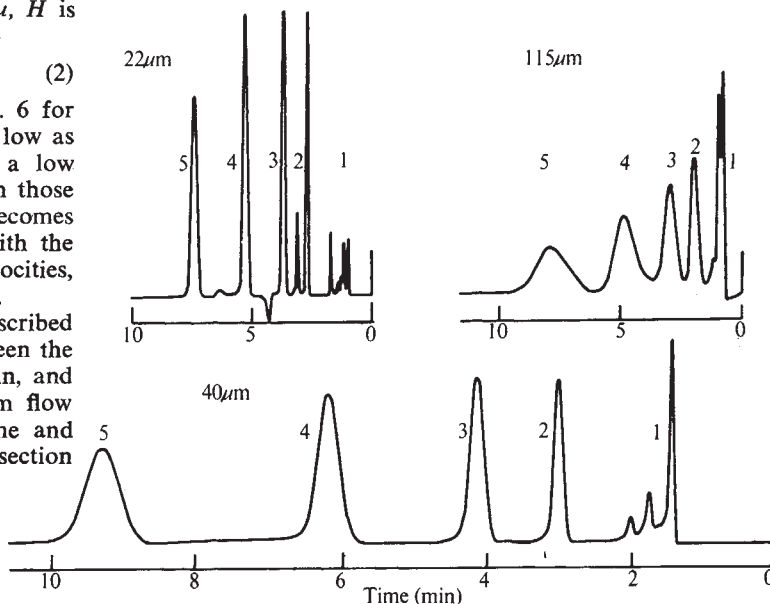


Fig. 7 Effect of particle size on resolution. Column length, 1 m; elution time for last peak, 7 to 10 min; stationary phase, BOP on 'Zipax'; mobile phase, cyclohexane; sample, about $10 \mu\text{g}$. 1 = Unretained solute; 2 = 2,4,xyleneol; 3 = *o*-cresol; 4 = *m*-cresol; 5 = phenol.

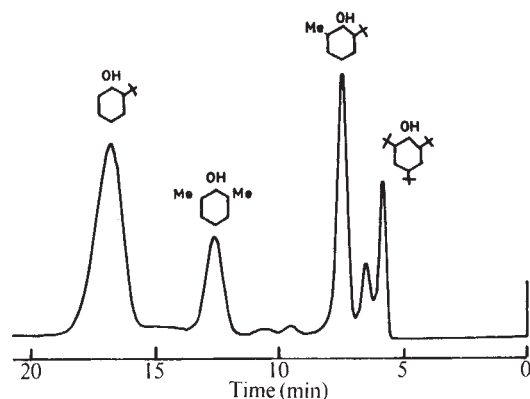


Fig. 8 Separation of antioxidants by adsorption chromatography on 'Corasil II'. Column length, 500 mm; particle size, 44 to 53 μ m; mobile phase, cyclohexane; —+, t-butyl group.

for the detection and injection devices would have to be so minute as to be beyond the reach of present day technology. The practical limit is likely to be reached with particles of 5 to 10 μ m. These would give high resolution analyses in 1 min or so with columns of reasonable length (100 to 500 mm). Detectors would not then have to be very much smaller than at present. An improvement of about ten-fold in performance should be possible in the quite near future.

Table 1 Calculated Elution Times for 'Zipax' of Different Particle Sizes using a Pressure Drop of 3,000 Pound Inch⁻²

Particle diameter (μ m)	Column length (mm)	Analysis time (s)
0.2	7	40
0.25	3	5
0.5	5	4
1	14	7
2.5	50	15
5	140	30
10	370	50
25	1,400	110
50	3,900	220
100	10,300	400

The theory outlined in this article also sets out guidelines for the optimum configuration for a packing material; it clearly indicates that conditions should be chosen so that both counter-dispersion mechanisms are effective in reducing peak spreading. In the flowing parts of the mobile phase both the tortuous flow and molecular diffusion counter the dispersive tendency, but in the stagnant regions only molecular diffusion operates and this mechanism becomes increasingly ineffectual as the flow rate increases (see Fig. 5). In the ideal packing the stagnant regions are therefore minimized, and this explains why the gradients of the plate height curves for porous materials shown in Fig. 6 are greater than those for surface layered particles.

The curve in Fig. 6 for 'Chromosorb' (a well known support popular in gas chromatography) is interesting, for it exhibits a large plate height but a low gradient. Although 'Chromosorb' is a porous material, and so might be expected to give a steeper dependence like 'Porasil', it is very difficult to pack, and the chief cause of its poor performance is not particle porosity but the poor geometry of the interparticle space which probably leads to channelling and other undesirable flow characteristics.

The most sophisticated support at present available for HPLC is probably 'Zipax', which was invented by Kirkland¹². It consists of small glass spheres which are coated with microspheres of silica deposited from a silica sol. By suitable control of conditions, successive layers of silica are deposited and finally bound to the glass surface. The final result is a bead covered with a hard porous layer about

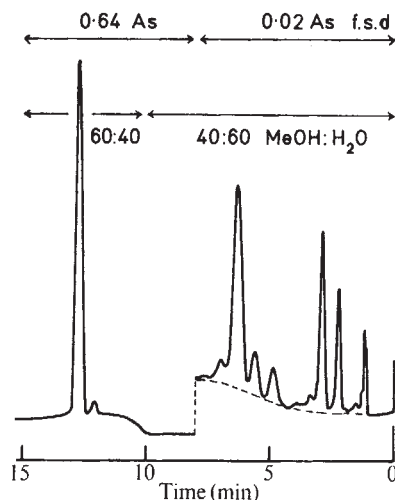


Fig. 9 Analysis of 'Trithion' on 'Permaphase'. Column length, 1 m; stationary phase, octadecyl silane chemically bonded to support.

1 μ m thick which can retain up to 1% by weight of stationary phase. An adsorptive material of similar configuration is Waters Associates' 'Corasil'²⁰ in which the bead is coated with a layer of silica gel with fine pores. This material is useful for separating substances which would normally be separated by thin layer chromatography. Fig. 8 shows the separation of some antioxidants on this material. An earlier approach to surface layered beads is that of Horvath, Preiss and Lipsky²¹, who coated solid glass beads with a thin layer of ion exchange resin. This material offered a significant improvement in the speed of analysis of nucleotides, but it has been overtaken in speed by 'Zipax' coated with ion exchange resin²².

An important technique in classical liquid adsorption chromatography was gradient elution or solvent programming. This technique has been applied with success in HPLC and is the analogue of temperature programming in gas chromatography. In gradient elution the composition of the solvent is changed either gradually or stepwise during the analysis of the sample; mixtures containing substances of a wide range of polarity can then be separated in a single analysis with good resolution at all points of the chromatogram²³. Until recently gradient elution was restricted to adsorption and ion exchange chromatography because it is essential that the stationary phase be insoluble in the mobile phase. Its range has now been greatly extended by the invention of partitioning materials in which the stationary phase is chemically bonded to the support²⁴. The use of such a material for a solvent programmed separation is shown in Fig. 9. The chromatogram of the commercial insecticide 'Trithion' shows one major component and several minor ones. As in gas chromatography, the identification of eluted fractions is an important part of a complete

Table 2 Formula of Trithion and Masses of Possible Fragments

		$\begin{array}{c} \text{S} \\ \\ \text{C}_2\text{H}_5\text{O}-\text{P}-\text{S}-\text{CH}_2-\text{S}-\text{C}_6\text{H}_4-\text{Cl} \\ \\ \text{H}-\text{C}_2\text{H}_4\text{O} \end{array}$					
Cumulative mass number from left	45	153*	185	199*	231	307	342* 344*
Cumulative mass number from right	342* 344*	297* 299*	296* 298*	189* 191*	157* 159*	143* 145*	111 113
							35 37

* These masses are those actually recorded.

analysis. When using liquid chromatography this is readily achieved by mass spectrometry. The eluent fraction containing the appropriate peak is collected and most of the solvent evaporated. About 5 μ l. of the residue is then taken up on a quartz sinter attached to the direct injection probe of the mass spectrometer (in our case an AEI model MS 902). The remaining solvent is evaporated in air and the probe inserted into the instrument. Identification of the principal components from a single chromatographic run is then possible. In this way the chief peak in Fig. 9 was conclusively identified as 'Trithion'. The formula of the compound and the masses of possible fragments are shown in Table 2.

State of the Art and the Future

The most important features of the new form of liquid chromatography are its speed and versatility. Although HPLC has existed only for a few years a large number of different types of compound have been separated. Some idea of their range is given by Table 3 which lists a selection of applications. Most of them have been carried out on surface layered materials, and in all cases good resolution was obtained in less than 20 min and sometimes in as little as 1 or 2 min. Sample sizes were generally between 1 and 50 μ g. Clearly sample type is no limitation, and the fact that polymers can readily be separated by gel chromatography shows that, by contrast with gas chromatography, HPLC is not limited by molecular weight.

Table 3 Some Classes of Compounds Analysed by HPLC

Simple aromatic compounds:

Phenyl alcohols, alkyl substituted phenols, amines, pyridines and quinolines, nitro benzene and derivatives, benzoic acids, phthalic acids and derivatives, sulphur and selenium heterocyclic compounds, anthraquinone derivatives, polynuclear aromatic compounds.

Antioxidants:

Amines, *t*-butyl phenols, polyhydric phenols and hydroquinones.

Plasticizers:

Long chain phthalate esters.

Pesticides:

DDT, 'Aldrin', EPN, carbaryl, 'Abate', phosphorus insecticides.

Herbicides:

Phenoxyacetic acid derivatives, 'Tandex', and other urca herbicides.

Drugs, analgesics and antibiotics:

Aspirin, caffeine, sulphonamides, penicillins.

Biochemicals and natural products:

Steroids, nucleic acid bases, ribonucleosides, alkaloids.

Undoubtedly the principal areas where improvements will be made are in column packings and detectors. Detector technology is still limited, for the only commercial detectors which are widely used and reliable are the ultraviolet photometer and the RI monitor; the last of these is barely sensitive enough for other than major component analysis. It seems likely that all highly sensitive detectors will have to be selective, but then many applications will best be served by specific detectors. The most likely candidates for the immediate future are the fluorometer and the polarograph, both of which have sensitivities comparable with the ultraviolet photometer for those substances to which they respond, but transport detectors of novel design and with facilities for detection of specific elements look most attractive if the present mechanical problems can be solved.

In column technology the further development of chemically bonded stationary phases with a wide range of functionality will have an important influence on the progress of HPLC, and for the time being surface layered materials will predominate. As the particle size is reduced, however, one operates further down the plate height curve (Fig. 6), progressively nearer the minimum, and with 5 or 10 μ m particles much of the apparent advantage of the layered

supports disappears. Thus the future probably lies in the development of sophisticated porous materials based on silica or silica gel with particle diameters of 5 to 10 μ m.

A question that is often asked is whether the new form of liquid chromatography can be scaled to the preparative level. There seems no reason why this cannot be done. Indeed there is good evidence^{25,26} that very wide columns, in which the sample is injected centrally, can give higher efficiencies than conventional narrow columns. This is because lateral dispersion is slow and in a wide column a sample injected centrally may never reach the walls before it emerges from the column. This happens, for example, with a column of bore 10 mm and length 500 mm, filled with 50 μ m particles. Wall effects are absent, and, provided the packing is uniform, very high efficiency can be obtained. With existing 2 mm bore columns it is possible to elute 100 μ g without significant peak distortion. 2 cm bore columns can easily be envisaged, so charges of 10 mg can probably be separated without loss of resolution. By using the kind of repetitive injection systems common in preparative scale GC, the preparation of gram quantities of highly pure compounds by HPLC seems quite feasible.

Conclusions

High performance liquid chromatography is undoubtedly an analytical method for the future. In only three years it has demonstrated its power to analyse complex mixtures in fields such as drugs, pesticides, high molecular weight aromatics, plasticizers, antibiotics, organic chemicals generally, and biochemicals. It has all the advantages possessed by gas chromatography apart from price, but it does not require compounds to be volatilizable. Although its sensitivity is somewhat below that of GC, it is considerably more sensitive than GC was at such an early stage in its development, say, around 1956. The speeds which are currently being attained, although excellent, can probably be improved about ten-fold without any dramatic changes in technology, and such improvement may be confidently expected over the next few years. Within the same period automatic prep-scale liquid chromatographs should become available.

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Nucleotide Sequence of the Gene Coding for the Bacteriophage MS2 Coat Protein

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By characterization of fragments, isolated from a nuclease digest of MS2 RNA, the entire nucleotide sequence of the coat gene was established. A "flower"-like model is proposed for the secondary structure. The genetic code makes use of 49 different codons to specify the sequence of the 129 amino-acids long coat polypeptide.

DETAILED knowledge of the structure of a viral gene should provide a firmer basis for explaining the mechanism, specificity and regulation of translation. Because the genes of RNA bacteriophages are part of the viral genome, their structures may also have evolved for optimal functioning in replication and encapsulation.

The RNA of the closely related phages f2, MS2 and R17, and especially the cistron coding for the coat protein, has been used extensively for *in vitro* study of protein synthesis¹. ³²P-labelled bacteriophage MS2 RNA was promising as a material for investigating the primary structure of this viral messenger². By using limited enzymatic digestion of the RNA at low temperatures and separating the products on polyacrylamide gels, we isolated RNA-fragments of limited length, suitable for direct sequence analysis³. Sanger *et al.*⁴ were the first to link nucleotide sequences with specific functions in translation, isolating a "hairpin", 57 nucleotides long, from the R17 coat protein cistron, from which they could deduce a set of codons used in the genetic message. Argetsinger-Steitz⁵ identified a hairpin containing the initiating AUG-codon, while Nichols⁶ determined the nucleotide sequence of another hairpin, which contained the coat termination signal; information on two more hairpin-regions was also reported⁷. Working with the phage MS2, we identified and characterized similar coat cistron hairpins⁸. These sequences were extended⁹, and we can now present the entire sequence of the coat protein gene.

Pure Fragments from the Coat Gene

All RNA fragments used for sequencing the coat protein cistron originated from ribonuclease T₁ hydrolysis of uniformly labelled ³²P-MS2 RNA, and subsequent separation on neutral polyacrylamide gels. Two sets of conditions were used: 1° hydrolysis with 1 U of enzyme per 20 µg of RNA for 30 min at 0° C and separation on a 12% gel; and 2° with 1 U per 100 to 200 µg of RNA for 30 min at 0° C, followed by separation

on a 6% gel. Further details and typical patterns of both separations have been published before^{10,11}.

As the amino-acid sequence of the coat polypeptide is known¹²⁻¹⁵ (albeit incorrectly as it turned out), a nucleotide sequence can be deduced for this gene, leaving open the many

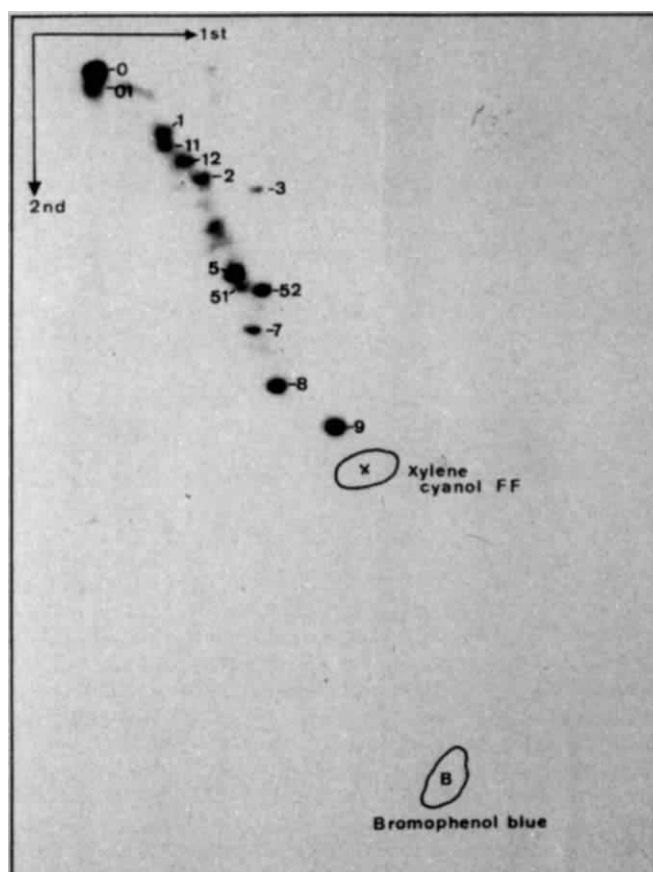


Fig. 1 Two-dimensional separation of band β_4 . The RNA of band β_4 was extracted and subjected to two-dimensional gel electrophoresis. The arrows indicate the directions of the run: the first dimension was on an 8% gel in 0.025 M citric acid + 6 M urea; subsequent separation was on a 16% gel in 0.04 M Tris-acetate, pH 8. The positions of the dye markers xylene cyanol FF (X) and bromophenol blue (B) are marked on the figure. Spots b_1 , b_2 and b_5 correspond to different regions of the coat protein cistron (Table 2). Spot b_{12} of this particular separation is a rarely observed fragment starting around the GGCGGA sequence (coding for amino-acids 13-14) and going till arrow 14 in Fig. 2.

positions which are not or only partly fixed due to the degeneracy of the genetic code. The information was sufficient to screen the relatively long oligonucleotides, obtained by complete ribonuclease T₁-hydrolysis of the gel bands and fingerprinting, and to see whether they could be derived from the coat gene. This resulted in isolation and sequencing of five fragments spread over the cistron⁸. A similar approach was used to detect regions from the coat protein cistron of R17^{4,6,7} and f2 RNA¹⁶.

Seeking longer fragments we examined slower moving bands in the 12% gel. But more important was a thorough analysis of the bands derived from the 6% gel (under the milder digestion conditions, known sequences were present in fragments of greater chain length). Each band was subjected to two-dimensional polyacrylamide gel electrophoresis¹⁷, involving an acidic gel system in the first dimension¹⁰ (10% or 8% gel), followed by a separation at neutral pH (20% or 16% gel respectively). Such a separation is shown in Fig. 1. After systematic T₁ mapping of all spots present in reasonable amounts, many fragments were recognized as being derived from the coat protein gene (Table 1).

Primary Structure Determination

We mainly used the sequencing methods developed by Sanger *et al.*^{4,19,20} for the analysis of purified fragments; details of these sequence determination studies will be published elsewhere.

We could not, however, build up the entire nucleotide sequence of the gene in this way. We ended up with four non-overlapping regions between the arrows 0-4, 4-14, 14-22 and 22-28 respectively (Table 1, Figs. 2 and 3). Although there is speculative evidence for their relative order we cannot exclude, purely on the basis of nucleotide sequence data, the possibility of extra nucleotides at three points (arrows 4, 14 and 22). The direct linkage is mainly based on the amino-acid sequence of the MS2 phage^{14,15}, which has recently been completed (Vandekerckhove and Van Montagu, personal communication). The nucleotide sequence obtained is shown in Fig. 4, together with the amino-acid sequence for which it codes. It comprises the ribosomal binding site for the coat protein cistron, the whole translated region, an intercistronic stretch of 36 nucleotides, and the ribosome attachment site of the RNA-polymerase gene⁵.

Before discussing other aspects, a few additional comments on the primary structure determination are appropriate. So far, the exact order of the T₁ oligonucleotides in the region between arrows 3 and 4 (coding for the amino-acids 7-18) has not yet been determined, since fragments containing this sequence were difficult to obtain in sufficient quantity. Nevertheless, the results are not compatible with the amino-acid sequence reported for the corresponding region in the f2 and R17 proteins^{12,13}. We considered three amino-acid changes to explain the nucleotide sequence data. These are situated

Table 1 RNA Fragments from MS2 Coat Protein Gene isolated from Limited Ribonuclease T₁ Hydrolysates

Fragment	Located between arrows No.		Chain length	Estimation of chain length		Yield
				12% gel	6% gel	
D6b11	22	24	30	26-31		(Irregularly found)
D5b5	2	3	31-32	29-35		Good
D5b3	5	7	30	30		Variable
D1z2; 85b0	24	25	43	40-50	40-54	Good (irregularly found)
84b2	0	3	± 52		46-52	Variable
C7z1	18	19	57	46-57		Moderate
{ C6r1; 84b2	13	14	32	49-61	46-62	Good; variable
{ C6r2; 84b7	14	15	27	27	27	Good; variable
{ C5z1; 83b2	8	10	61	52-64	51-66	Low (irregularly found)
{ C5z3	8	9	34	34		Good
{ C5z5	9	10	27	34		Good
82b7	1	4	78		63-82	Variable
82b4	18	20	72		72	(Irregularly found)
82b1	24	27	71		71	(Irregularly found)
C2b3	16 (15)	20	85 (87)	68-86		Variable
81b106	14	17	36		72-94	(Irregularly found)
81b2	22 (23)	25	73 (69-70)		73 (69-70)	Moderate
γ8b4	0	4	± 88		86-112	Variable
B13b1; γ8b6	15	21	98	85-110	85-110	Moderate; good
γ8b3	22	26	89		89	Variable
γ6b1	22	27	101		100-132	(Irregularly found)
B4b5	14	20	114	128-172		(Irregularly found)
{ B2b23; γ4b168	12	14	37	150-200	150-200	Moderate
{ B2b22; γ4b16	11	14	40	40	40	Low
{ B2b11; γ4b4	14	21	125	125	25	Moderate
{ γ2b13	12	14	37		190-260	(Irregularly found)
{ γ2b4	14	22	141		141	(Irregularly found)
{ γ1b11	9	14	69		212-280	Good
{ γ1b7	14	21	125		125	Good
{ B7b14	9	14	69		240-330	Good
{ B7b7	14	21	125		125	Good
{ B7b6	14	22	141		141	Moderate
{ A3b7	14	21	125	245-335		(Irregularly found)
{ B5b91	8	14	103		295-400	(Irregularly found)
{ B5b52	14	21	125		125	(Irregularly found)
{ B4b4	6	14	138		340-450	Variable
{ B4b2	4	14	145		145	Good
{ B4b5	14	21	125		125	Good
{ B4b6	14	22	141		141	Variable
{ B4b1	22	28	± 180		± 180	Good

The nomenclature of the gel bands is described in reference 11. Bands from the 12% gel are referred to by Roman capitals, while Greek letters are used for bands of the 6% gel. Fragments already described earlier⁸ have been included for completeness; they were purified by homochromatography on thin layers¹⁸ (indicated as *t*), or by acidic gel electrophoresis¹⁰ (indicated as *z*). All other components were obtained after two-dimensional gel electrophoresis (indicated as *b*). The numbers of the arrows correspond to Fig. 2. Fragments taking part in a complex have been connected with hyphens, but not all components of such complexes are necessarily listed. The same components as found in β4 were also present in several slower moving bands (for example, β3, α3, α2 and α1).

at positions 11 (Asp), 12 (Asn) and 17 (Asp). Each difference can be accounted for by a single A $\leftrightarrow$ G transition in the codons AAPyp (Asn) $\leftrightarrow$ GAPyp (Asp). Recent amino-acid sequence studies of the MS2 coat protein (Vandekerckhove and Van Montagu, personal communication) have directly confirmed the changes which we propose. The coat protein of MS2 thus differs in three positions from R17 and in a fourth place from f2 (this being the only difference between R17 and f2). This agrees well with the serological data of Scott²¹, who showed that although MS2-R17-f2 were closely related, the former two were nevertheless not identical. We found this difficult to explain as their coat proteins, the most obvious candidate as antigenic determinant, were thought to be identical.

Repeated analysis of the fragment 14-22 revealed the presence of a heterogeneity in the region coding for amino-acid 109 (Gln). We found that in our RNA population both CAA and CAG codons were used (in a ratio of 2 to 1). Fragments containing the different sequences were sometimes separated (albeit incompletely) on the two-dimensional gel. Two T₁-maps of this sequence (where the alternatives appear as CAAG and CAG) are shown in Fig. 4. After returning to our original MS2 stock for phage growth², however, only CAA was found in that position.

Secondary and Tertiary Structure

Physico-chemical studies had revealed that phage RNAs contain a high degree of secondary structure^{22,23}. The helicity was estimated as 63% to 82%²⁴⁻²⁶. It was first shown for ribosomal RNA^{27,28} and subsequently for phage RNAs^{3,4,11} that limited treatment with nucleases at low temperature results in a characteristic non-random pattern of breakdown. This is generally believed to be indicative of a specific secondary and tertiary structure, although the presence of a limited number of different conformational states cannot be excluded. Alternative conformations have been described for tRNA²⁹ and there is some experimental evidence for phage RNA as well^{30,31}. We believe, however, that our

entire approach of isolating specific fragments from partial digests was successful only because the same secondary structural features were present in most if not all molecules of the population, and these partial digestion products are convenient starting material for the construction of a secondary structure model.

Extensive characterization of material present in the gel bands of both primary systems provided numerous examples for the importance of specific secondary binding forces between two (or more) RNA segments. In agreement with others^{32,33}, our results indicate that the mobility on a neutral gel is mainly a logarithmic function of the molecular weight. For a number of fragments, there is reasonable agreement between the actual chain length, known from the primary structure, and the estimate, based on the mobility in the gel (Table 1). In other cases there is clearly a large discrepancy, which can most easily be explained by assuming that they behaved in the neutral gel system as part of a tightly-bound complex, for example, band C5 contains the fragments z3 ($n=34$) and z5 ($n=27$) but also z1 ($n=61$). It should be noted here that the primary gel (either 12% or 6%) is run under neutral conditions, whereas the different systems used for the subsequent fractionation make use of denaturing conditions (low pH and urea). Slower moving bands from the 6% gel contain even more intricate complexes. For example, β_4 contains not only the fragments b2 and b5 linked by the interactions in region f, but also the fragment b1 occurs consistently in the same band. Its presence can most easily be explained by assuming that it is linked to the former two (and most likely to a segment of the sequence appearing simultaneously in the complex; Fig. 3). Together the three fragments comprise approximately 460 nucleotides, in agreement with the estimated chain length of β_4 (340-450).

The "Flower" Model

It seemed reasonable to start from the partial digestion products (and complexes) for the construction of a secondary

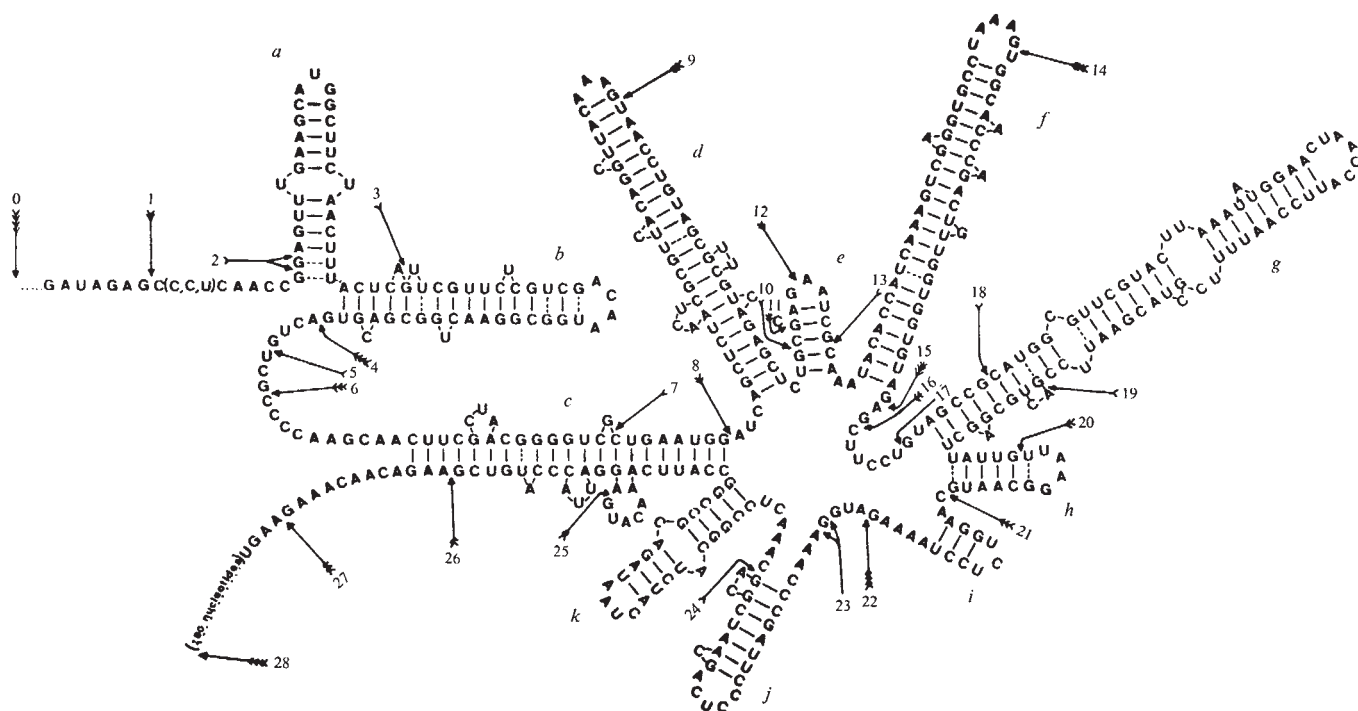


Fig. 2 A model for the secondary structure of the coat protein gene (the "Flower" model). Splitting points for T₁-ribonuclease observed in the partial digests are indicated by arrows. The number of feathers of the arrows give a semi-quantitative measure of the susceptibility of the Gp-N bond (arrows with no feathers point to occasionally found splitting points). Since a part of the sequence may exist in an alternative configuration (Fig. 6) some of the splitting points marked on this figure may in fact reflect the other structure (arrow 26). The different fragments isolated can be read from this figure in combination with the data summarized in Table 1. Base-paired regions are termed a to k. Table 2 lists the stability numbers of these regions, calculated according to Tinoco *et al.*³⁴.

structure model of the coat protein cistron. Beginning with the shortest fragments (Fig. 3) and optimizing secondary structure by also allowing G : U base pairs and slight deformations such as looped-out bases (bulges) and interior loops (bubbles), one can draw a series of models, which are similar in general outline but which vary in detail. Tinoco, Uhlenbeck and Levine³⁴ presented a simple method which permitted the estimation of the thermodynamic stability of various pairing schemes, and thus the choice of the most stable configuration. In this method, stabilizing and destabilizing features are expressed in quantitative, algebraic terms. On this basis, the model of the coat gene presented in Fig. 2 was derived. We pro-

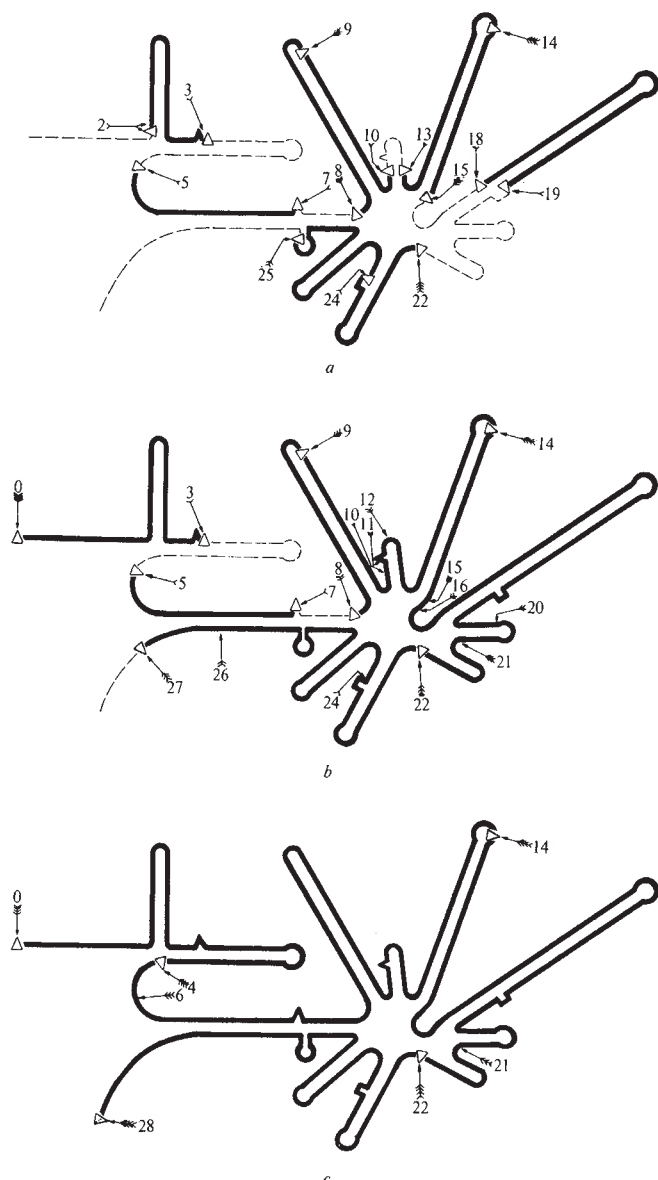


Fig. 3 Outline, illustrating different stages of hydrolysis. Fragments typical for a certain stage of breakdown are represented as a solid line. The model, and the arrows, correspond to Fig. 2. Both ends of a fragment are indicated by open triangles. Arrows not pointing to a triangle indicate positions of splitting in intermediate stages of hydrolysis. *a*, Shortest fragments isolated from different parts of the molecule (the region between arrows 5 and 7 forms a short hairpin in the alternative model, Fig. 6). *b*, Different intermediary stages of hydrolysis. The three hairpin-loops in the middle of the cistron appear as one complex. The beginning and the end of the cistron are also elongated. *c*, Finally, the whole region studied appears as four fragments. The beginning of the cistron (between arrows 0 and 4) is found independent from the others. The latter three fragments are present together, presumably as a complex, in bands β_4 , β_3 , α_3 , α_2 and α_1 (two of these fragments can occur in slightly varying length).

GCU·UCU·AAC·UUU·ACU·CAG·UUC·GUU·CUC·GUC·GAC·AAU·GGC·GGA·ACU·GGC·GAC·GUG·ACU·GUC·GCC·CCA·AGC·AAC·UUC·Ala Ser Asn Phe Thr Gln Phe Val Leu Val Asp Asn Gly Thr Gly Thr Val Ala Pro Ser Asn Phe	1	5	10	15	20	25
GCU·AAC·GGG·GUC·GCU·GAA·UGG·AUC·AGC·UCU·AAC·UCG·CGU·UCA·CAG·GCU·UAC·AAA·GUA·ACC·UGU·AGC·GUU·CGU·CAG·Ala Asn Gly Val Ala Gln Trp Ile Ser Ser Asn Ser Arg Ser Gln Ala Tyr Lys Val Thr Cys Ser Val Arg Gln	30	35	40	45	50	55
AGC·UCU·GCG·CAG·AAU·CGC·AAA·UAC·ACC·AUC·AUC·AAA·GUC·GAG·GUG·CCU·AAA·GUG·GCA·ACC·CAG·ACU·GUU·GGU·GGU·GUA·Ser Ala Gln Asn Arg Lys Tyr Thr Ile Lys Lys Val Glu Val Pro Lys Val Ala Thr Gln Thr Val Gly Gly Val	60	65	70	75	80	85
GAG·CUU·CCU·GUA·GCC·GCA·UGG·CGU·UCG·UAC·UUA·AAU·AUG·GAA·CUA·ACC·AUU·CCA·AUU·UUC·GCU·ACG·AAU·UCC·GAC·Glu Leu Pro Val Ala Ala Trp Arg Ser Tyr Leu Asn Met Glu Leu Thr Ile Pro Ile Phe Ala Thr Asn Ser	90	95	100	105	110	115
UGC·GAG·CUU·AUU·GUU·AAG·GCA·AUG·CAA·GGU·CUC·CUA·AAA·GAU·GGA·AAC·CCG·AUU·CCC·UCA·GCA·AUC·GCA·AAC·Cys Glu Ile Val Ile Val Lys Ala Met Gln Gly Leu Leu Lys Asp Gly Asn Pro Ile Pro Ser Ala Ile Ala Ala Asn	120	125	130	135	140	145
UCC·GGC·AUC·UAC·UAA·UAG·ACG·CCG·GCC·GCC·AUU·CAA·ACA·UGA·GGA·UUA·CCC·AUG·UCG·AAG·ACA·ACA·AAG·AAG·(U) Ser Gly Ile Tyr	150	155	160	165	170	175

Fig. 4 Nucleotide sequence of the coat protein gene, together with the amino-acid sequence it specifies. The gene is preceded and followed by untranslated intergenic regions. The numbers refer to the position of the amino-acid residues in the coat protein (1-129) and in the polymerase molecule (1-6).

pose to call it the "flower" model, as one can discern a stalk with petals. 66.4% of the nucleotides are involved in helical regions.

The stability constants of the various base-paired segments are summarized in Table 2. No alternative structures with comparable stability could be obtained in most parts of the sequence, except for the regions between arrows 15 and 18 and between 19 and 22 (or even 24); these parts of the structure should therefore be considered as highly tentative. Another part of the sequence can clearly be represented in two different ways, either as written as in Fig. 2 (stem *c* of the figure), or alternatively as a structure with two hairpin-loops and a short stem as shown in Fig. 6. This model has the same overall stability (Table 2).

In general, the position of the splitting points (arrows in Fig. 2) confirms the proposed secondary structure. Four-feather arrows point to a G-residue in a loop at the top of a hairpin (arrow 14) or in single-stranded regions (arrows 4 and 22). Many other arrows point to G-residues which either lie in single-stranded regions or adjacent to the end of a hairpin or to looped-out bases, whereas almost every G-residue in a double-stranded region is not split by the enzyme.

In constructing the model, many choices were based on the stability constants as defined by Tinoco, Uhlenbeck and Levine³⁴. As these authors point out, the theoretical evaluation of the stability of polynucleotide conformations will certainly be refined in the future. We believe, however, that these improvements will mainly involve details rather than the general outline of the proposed model, as at least a large part is experimentally supported by several lines of evidence. Another theoretical improvement will be the construction of one or more secondary structures by computer evaluation of the primary structure. This, however, should preferably be done after the entire viral nucleotide sequence is known and perhaps also after the program has been refined. In addition,

experimental approaches will undoubtedly contribute to a better understanding of the polynucleotide conformation.

Finally, it should be stressed that the "flower" model, as presented in Fig. 2, is presumably further folded in an intricate, three-dimensional superstructure. This may explain why some G-residues, apparently situated in single-stranded regions, are not, or only rarely, split.

Biological Implications

J. Argetsinger-Steitz⁵ determined the nucleotide sequence of the region, surrounding the initiating AUG for each of the three R17 cistrons. Part of the MS2 A-protein ribosome binding site has previously been sequenced¹¹. We now find that the ribosome binding regions for the MS2 coat cistron and the MS2 RNA-polymerase cistron are virtually identical to their R17 counterparts (only a G to A change is noted in the former region; a similar base change, however, was found by Cory, Spahr and Adams³⁵ for R17 RNA and by Gupta *et al.*³⁶ for f2 RNA). So the untranslated regions, preceding the initiating AUG-codons, seem to be genetically conserved.

The secondary-structure model, however, does not provide a clear clue as to what constitutes a ribosomal binding site. The first AUG of the coat cistron is conveniently located on top of a hairpin⁵. But no such structure can be proposed for the first AUG of the RNA-polymerase cistron; even in the alternative model (Fig. 6), this AUG is buried by base-pairing. On the other hand, several AUGs seem to be located in single-stranded regions (for example, one between the hairpins *i* and *j*, and another in the untranslated region preceding the RNA-polymerase cistron). Their inability to bind ribosomes may perhaps be explained by their being buried in a three-dimensional folding.

The nucleotide triplets, which code for the 129 amino-acids long coat polypeptide, are listed in Table 3. Not unexpectedly,

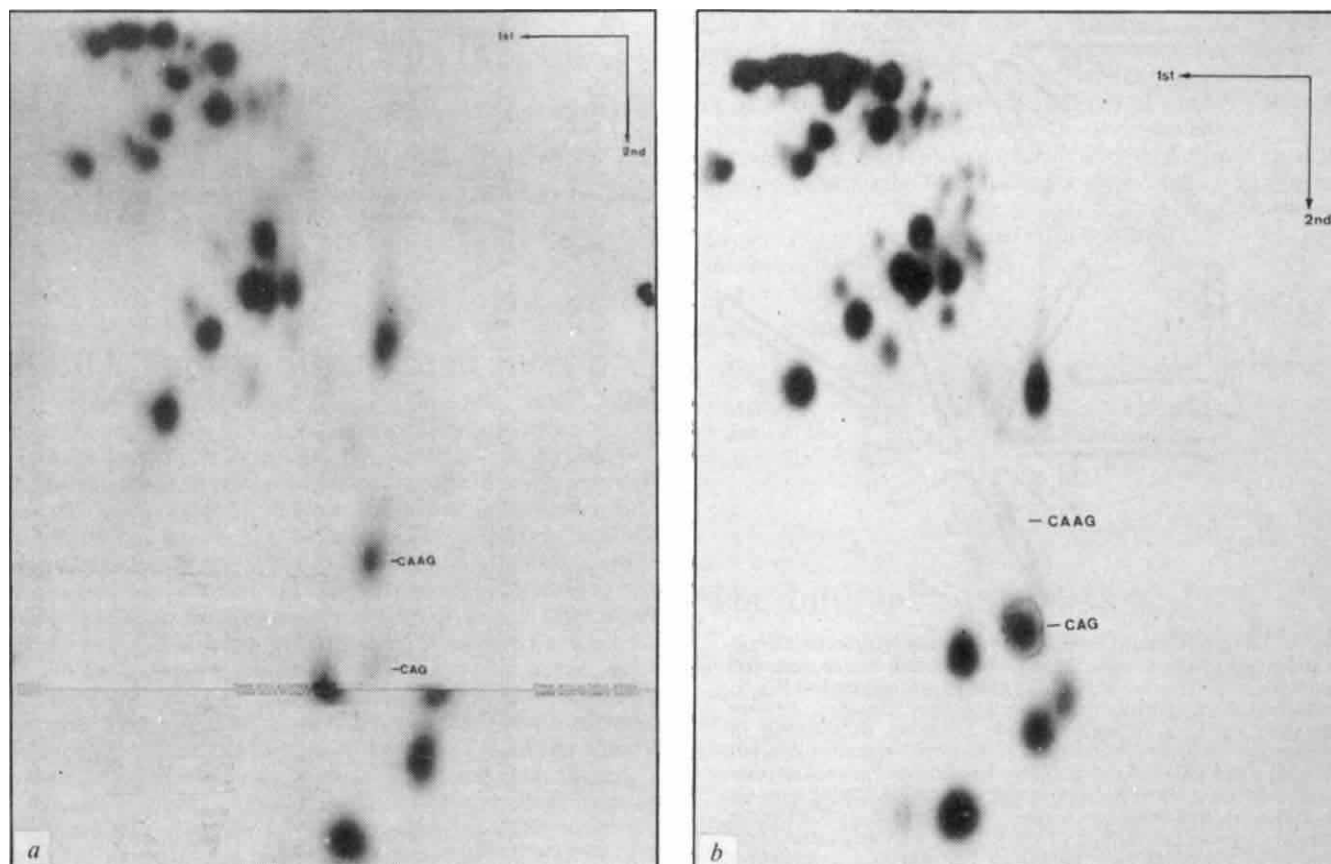


Fig. 5 Ribonuclease T₁ fingerprints of the fragment between arrows 14 and 22 (Fig. 2), illustrating the observed sequence heterogeneity in this region. *a*, Fragment in which the sequence CAA-G (coding for amino-acid 109; Gln) is predominant; *b*, sequence "variant" separated from the former by two-dimensional gel electrophoresis. Spot CAAG is replaced here almost completely by CAG (and G) (such fragments were no longer observed after returning to our original phage stock).

Table 2 Stability Numbers of Base-paired Regions	
Region	Stability number
a	4
b	6
c	10
d	16
e	1
f	19
g	13
h	-1
i	0
j	1
k	5
l	4
m	3
n	3

The stability numbers were calculated by the method of Tinoco *et al.*³⁴. The base-paired regions are indicated in Fig. 2 (a-k) and in Fig. 6 (l-n). The latter figure represents an alternative secondary structure model for roughly that part of the molecule involved in the base-paired region c (Fig. 2). Stable secondary structures have positive values, negative values characterize structures unstable with respect to the single strand (region h is nevertheless represented as a double-stranded structure in Fig. 2, because of our data on the nature of the fragments obtained).

our results fully confirm, in a most straightforward way, the genetic code word dictionary compiled by Ochoa-Nirenberg-Khorana. In all, 49 different code words are used. For a few amino-acids the choice between the degenerate codons seems to be non-random, although this could still be a coincidence. Several places in the code table remain unoccupied, and it seems unlikely that chance alone can explain this. Especially noteworthy is the absence of AUA as a codon for Ile and UAU as a codon for Tyr; these results are corroborated by the preliminary information on codons used in the polymerase cistron⁹.

Although in some cases the choice between degenerate code words may be dictated by the properties of the *Escherichia coli* translation machinery, in other instances it may be based on secondary structure requirements for the viral RNA. In fact, there is (statistically weak) evidence supporting this assumption.

Table 3 Codons found in the MS ₂ Coat Protein Gene					
	U	C	A	G	
U	Phe { ° °°°	Ser { °°° °°	Tyr { °°°°	Cys { ° °	U
					C
	Leu { ° °°	°°	Ochre○ Amber○	Opal Trp °°	A G
C	Leu { °° °°	Pro { °° °°	His { °° °°	Arg { °°° °	U C
					A
	°°°°	°°°°	Gln { °°°°°	°	G
A	Ile { °°°° °°°°	Thr { °°°° °°°°	Asn { °°°°° °°°°°°	Ser { °°°° °°°°	U C
					A
	Met ○°°	°	Lys { °°°°° °	Arg { °°° °	G
G	Val { °°°° °°°	Ala { °°°°° °°°°°°	Asp { °°°° °°°	Gly { °°° °°°	U C
					A
	°°°	°	Glu { °°° °°°	°	G

The Polarity Effect

It is known, both from *in vivo* and *in vitro* results, that at least part of the coat gene has to be translated in order to allow translation of the following RNA polymerase cistron³⁷⁻⁴⁰. Coat amber mutants at position 6 are strongly polar under non-permissive conditions; similar mutants at positions 50, 54 or 70, however, are not polar. A logical explanation is that polarity is relieved when ribosomes travel over the region somewhere between positions 6 and 50 of the coat cistron.

This hypothesis can now be explained in molecular detail by the "flower" model. Indeed, when ribosomes translate the region of amino-acids 24 to 32 of the coat cistron, they have to open up the hairpin region c. In this way they release the opposing strand, and, conceivably, this is sufficient for other ribosomes to bind at the RNA-polymerase-initiating AUG.

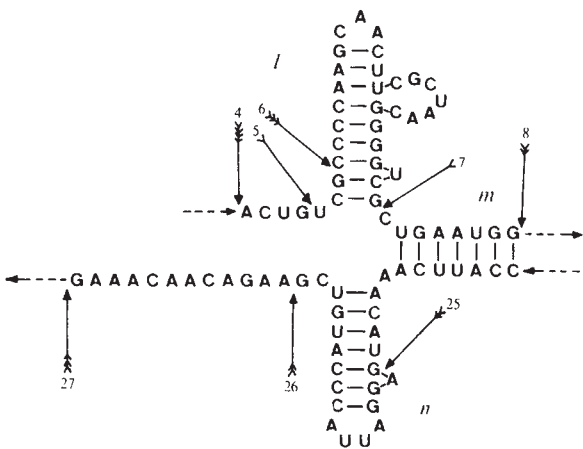


Fig. 6 Alternative model for a part of the coat protein gene and for the initiation site of the polymerase gene. Base-paired regions are termed l to n. The initiating AUG-triplet of the polymerase cistron is present in the CCC.AUG.U sequence of region n. For further explanation see legend to Fig. 2.

It is known that the viral RNA can adopt a conformation which does not show the polarity effect³¹. This can perhaps be explained by the alternative model, shown in Fig. 6.

Specificity of Mutagenesis

If transitions alone are legitimate, then amber codons (UAG) could arise from Gln codons (CAG) or from Trp codons (UGG). Zinder and Cooper⁴¹ isolated a series of amber suppressor-sensitive mutants of f2 by treatment with nitrous acid. Some of these mutations were in the coat cistron, and their location was shown by amino-acid sequence analysis⁴² to be either at position 6 (Gln) or position 70 (Gln). A collection of similar amber mutants from the phage R17 was isolated by using nitrous acid and fluorouracil as mutagens^{38,43}. These coat mutants corresponded to positions 6, 50 or 54 (all Gln). Using nitrous acid as well as hydroxylamine, Van Montagu (refs. 44, 45 and personal communication) isolated and characterized a series of MS2 amber mutants. The nitrous acid induced coat mutants were at position 6 (the major fraction) and at position 50, while the hydroxylamine induced coat mutations were localized at positions 50, 70 and 82 (the latter is the only known Trp-mutation). It is of interest to examine these mutation data in the light of the structural model. The CAG (Gln) codons at positions 6, 50, 54 and 70, where mutations can occur, are all involved in a discontinuity of the double-stranded hairpin stem (an A or C residue is looped out). The CAG (Gln) codon at position 40, where no mutation has ever been found, forms part of an uninterrupted bihelical segment (either the mutagens do not attack

this C, or else base-pairing in this region is critical and an induced mutation in the opposite strand would give rise to an unacceptable missense, for example, a UGU Cys to UAU Tyr). The only other Gln-residue in the sequence, at position 109, is coded by a CAA-codon, which would result in a non-viable ochre mutation (although in some of our stocks a CAG-variety at this position was present). Finally, the UGG-codon which was mutated by hydroxylamine treatment to UAG is also found in a double-stranded region.

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Outer Spiral Structure and High Velocity Clouds in the Galaxy

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New observations of high velocity clouds show that these may be interpreted in terms of an extension of the outermost spiral arm, confirming and extending the model of Habing.

THE high velocity clouds (HVCs) found in neutral hydrogen surveys of the Galaxy provide one of the enigmas of galactic

astronomy. These clouds are found predominantly with negative (approach) velocities, at least in the parts of the sky accessible from northern observatories, and they are concentrated mostly in the region of galactic longitudes $l =$ between 60° and 180° and latitudes b between $+10^\circ$ and $+60^\circ$. A few positive velocity (recession) clouds have been detected; they are found chiefly at $l > 22^\circ$. The distribution over the sky of the known HVCs is shown in Fig. 1. The available information on the position, velocity and structure of individual clouds has inspired various explanations of the HVC phenomenon. After summarizing existing observations and theories I suggest that a more comprehensive

explanation of the observations can now be made, based on new data.

Habing¹ gave three alternative explanations of the HVCs. He favoured the proposal that the HVCs were closely related to the outer arm, suggesting they were an extension or halo of that arm. The HVCs were displaced above the galactic plane like the outer arm at $l=120^\circ$, and they have similar radial velocities. He therefore proposed that the HVCs, like the outer arms of the Galaxy, may have been displaced from the galactic plane by the gravitational influence of the Magellanic Clouds. Habing's second suggestion, which he found less attractive, was that the HVCs were parts of a large complex at a distance of 100 to 300 pc falling towards the Sun. His third proposal was that the HVCs were a cloudbank situated 1 to 2 kpc from the Sun and falling into the Galaxy in the manner envisaged by Oort². Habing felt that only those HVCs at intermediate latitudes which were separated from most of the HVCs near the plane required the third explanation.

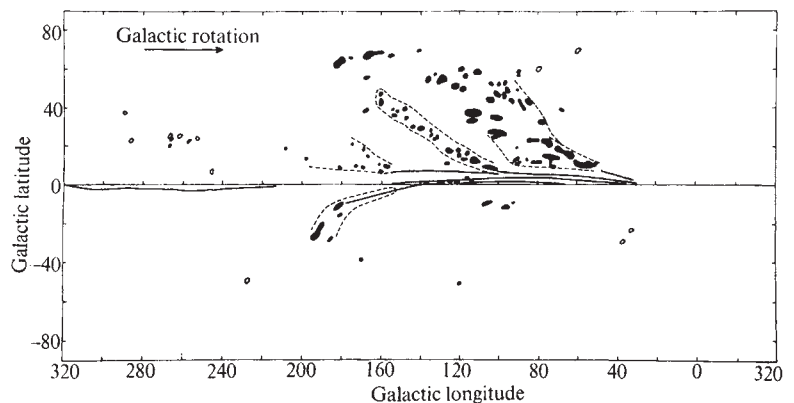
It has also been suggested that the HVCs are extragalactic objects. Kerr and Sullivan³ proposed that the HVCs might be moving in eccentric orbits around the Galaxy at distances of ~ 50 kpc. Verschuur⁴ argued that the HVCs would be gravitationally stable objects if they were several hundred

proposal. The observations demonstrate the existence of spiral arms further from the galactic centre than the outer arm. Some of these arms have already been detected in an intermediate latitude survey of limited extent by Kepner⁷. Another significant observation is that the bulk of the high velocity material is closely associated with these new outer arms, and, in addition, it follows the well known tilt of the Galaxy. The small fraction of the high velocity material which has a distribution of velocities incompatible with galactic rotation is readily understood on the present picture, in terms of the infall of gas which has broken away from the outer arms in the cosmic event which caused the tilt in the galactic plane. Nothing outside the Galaxy, such as extragalactic satellites or infalling intergalactic material, is required. I present here a summary of the evidence which leads to this picture of the high velocity material. A detailed analysis will be published elsewhere.

Observations

The Mark II radio telescope (beamwidth 31×33 arc min) was used with a parametric amplifier and autocorrelation spectrometer⁸ to observe a bandwidth of $1,000 \text{ km s}^{-1}$ at a velocity resolution of 7.4 km s^{-1} . Baselines could be estab-

Fig. 1 The distribution of high velocity clouds (HVCs) in galactic coordinates. Negative velocity HVCs are shown as filled areas; positive velocity HVCs are shown as open areas. Connected groups of HVCs are shown bounded by broken lines.



kpc distant; so they could be independent members of the Local Group. But subsequent observations have shown that most HVCs are connected to the outer spiral structure and that therefore they cannot be extragalactic objects.

Oort⁵ suggested two possible origins of the infalling gas which he believes are responsible for the HVCs. The first proposition, which, he concludes, meets insurmountable difficulties, is that the gas was ejected from the galactic nucleus and is now raining down onto the galactic plane. He favours the idea that the infalling gas is the remnant of the cosmic material which formed the Galaxy. Dieter⁶ noted, in high sensitivity observations, that the HVCs are continuous in velocity and position with the outer arm of the Galaxy. Moreover, she found many more low density HVCs. She pictured the high velocity gas as a ring of material falling radially onto the periphery of the Galaxy at distances of 12 to 16 kpc from the galactic centre. This follows Oort's model in adopting an origin outside the Galaxy for the infalling gas.

A high sensitivity survey of the outer spiral arms and HVCs has now been made at Jodrell Bank, and the results are presented here. These lead to a unified picture of the spiral arms and HVCs which is an extension of Habing's

proposal. The observations demonstrate the existence of spiral arms further from the galactic centre than the outer arm. Some of these arms have already been detected in an intermediate latitude survey of limited extent by Kepner⁷. Another significant observation is that the bulk of the high velocity material is closely associated with these new outer arms, and, in addition, it follows the well known tilt of the Galaxy. The small fraction of the high velocity material which has a distribution of velocities incompatible with galactic rotation is readily understood on the present picture, in terms of the infall of gas which has broken away from the outer arms in the cosmic event which caused the tilt in the galactic plane. Nothing outside the Galaxy, such as extragalactic satellites or infalling intergalactic material, is required. I present here a summary of the evidence which leads to this picture of the high velocity material. A detailed analysis will be published elsewhere.

In places the outer spiral structure was identified as separate high velocity features; elsewhere it blends with stronger features on the spectra making up the survey. The reality of any spiral arm can be demonstrated by plotting its intensity as a function of latitude. Fig. 2 shows the latitude distribution across several spiral arms at $l=120^\circ$ and 230° . In the $l=120^\circ$ cut, all the arms are seen to extend further above the plane than below, in the manner expected from the known tilt of the galactic plane in this region. The far arm extension (feature 0* of ref. 7) has its principal maximum on the galactic plane and an extended maximum, at $b \sim +16^\circ$; it can be traced up to $b = +26^\circ$. Similarly, arm X has its maximum at $b = +6^\circ$ and extends up to

$b = +20^\circ$. At $l = 120^\circ$ the latitude band from $b = +10^\circ$ to $+20^\circ$ contains some HVCs (see Fig. 1) in the velocity range -100 to -150 km s^{-1} . With the higher sensitivity of these observations it is clear that these HVCs are peaks in extended arm-like features running at a small angle to the galactic plane. A similar situation is illustrated in the $l = 230^\circ$ cut, where the outer regions of the Galaxy are seen at positive velocities. At this longitude, the tilt of the galactic plane to negative latitudes is beginning to show. The high (positive) velocity gas can also be seen well away from the galactic plane in all the arms—with a somewhat greater extension to higher negative latitudes. The irregular structure of this neutral hydrogen distribution is identical to that seen at negative velocities at $l = 120^\circ$. These irregular features could equally well be described as HVCs at positive velocities. I emphasize that the structure shown in these sections is real—it is seen in independent spectra taken at

sions of the previously identified spiral arms. The actual location of the spiral arms depends on the rotation model chosen, but the Schmidt model is not likely to be seriously in error, and consequently the positions allocated here are realistic, indicating the existence of spiral structure out to $\sim 20 \text{ kpc}$ from the galactic centre. The widespread distribution of this structure around the outer regions of the Galaxy also indicates that the positions derived are not the result of some local systematic distortion of the velocity field.

The neutral hydrogen line integral through the outer arms is typically $2\text{--}5 \times 10^{19} \text{ cm}^{-2}$. This would give a space density of $1\text{--}2 \times 10^{-2} \text{ cm}^{-3}$ for an arm depth of 1 kpc in the line of sight. These values are roughly 1% of those normally found in the brighter arms such as the Orion and Perseus arms. The displacement of the main ridge of these arms above the galactic plane at $l = 60^\circ$ to 120° is $1\text{--}2 \text{ kpc}$. The extreme extensions of the arms out of the plane can be

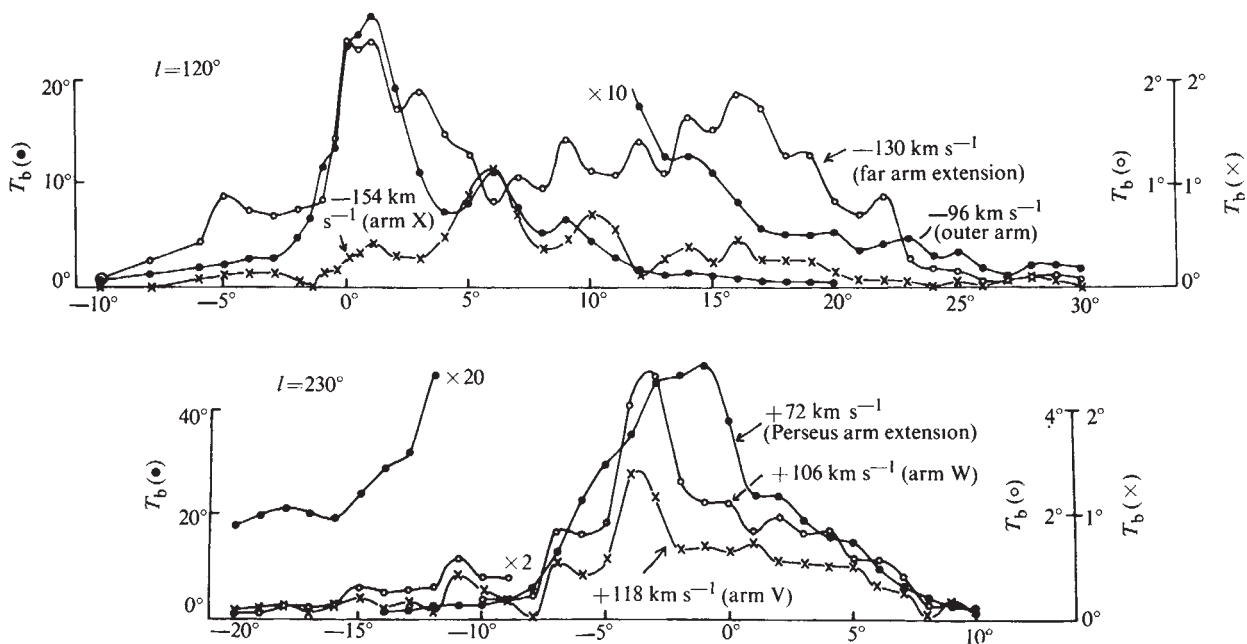


Fig. 2 The latitude distribution of brightness temperature across spiral arms at $l = 120^\circ$ and 230° . The temperature scale for each arm is indicated. Note that there is a scale change in the high latitude part of the plot of the bright arm to enable it to be compared more directly with the faint outer arms.

Jodrell Bank and is confirmed in the recent data published by Dieter⁹, which were taken on a coarser latitude grid with apparently rather less sensitivity.

The position of the outer spiral arms can be determined on the assumption that they are in circular motion in the Galaxy and that they obey the Schmidt¹⁰ rotation model. The location relative to the established spiral arms is shown in Fig. 3. The data used for the latter arms were those published by Weaver¹¹, because they were taken with a similar angular resolution to the present material; in principle any of the other interpretations of the velocity data (see, for example, ref. 12) could have been used. The weaker spectral features described here define spiral structure in the outer part of the Galaxy which is as convincing as the inner structure. It is interesting that the arm separation is still $2\text{--}3 \text{ kpc}$ and that the outermost arms, like the inner arms, also appear to be trailing. Further evidence of their reality is provided by the gradual fall in density in the trailing direction and, moreover, it seems that some of the arms, such as the far arm extension and arm X, are outer exten-

traced to $4\text{--}5 \text{ kpc}$ above the plane and $\sim 1 \text{ kpc}$ below the plane.

High Velocity Clouds

The close association of the HVCs with the outer spiral structure is clear from this and other high sensitivity studies. With the identification of new arms at even higher negative (and positive) velocities than found in the brighter arms, the range of velocities found in HVCs is covered by the velocity dispersion in these new arms. Furthermore, the HVCs are observed to lie in continuous bands of emission emerging at shallow angles from the plane. The band of HVCs that leaves the plane at $l \sim 100^\circ$, $b \sim +7^\circ$ has already been recognized by Meng and Kraus¹³ and by Hulsbosch¹⁴ and is clearly demonstrated in the $l = 120^\circ$ cut in Fig. 2. Four such bands of HVCs have been identified in these observations (Fig. 1). They all emerge from the galactic plane in a trailing sense. Very few ($\sim 10\%$) of the HVCs plotted in Fig. 1 do not belong to these bands. The bright-

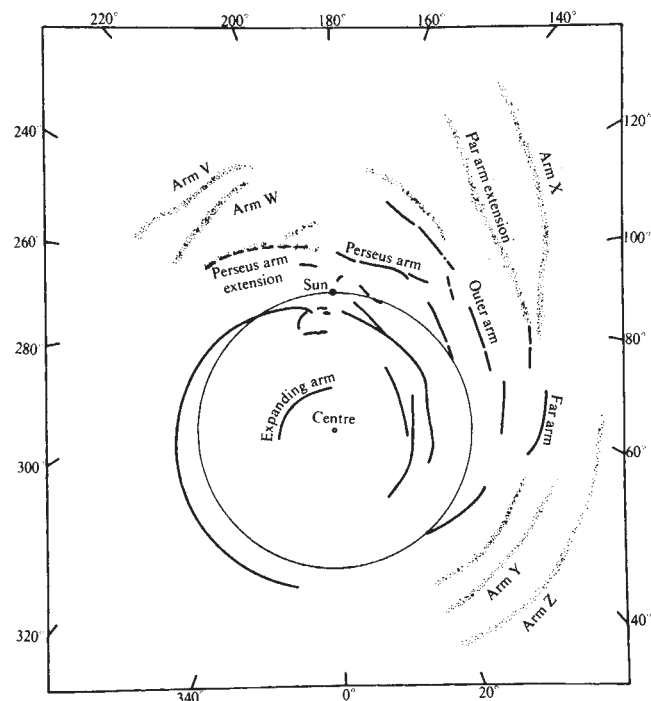
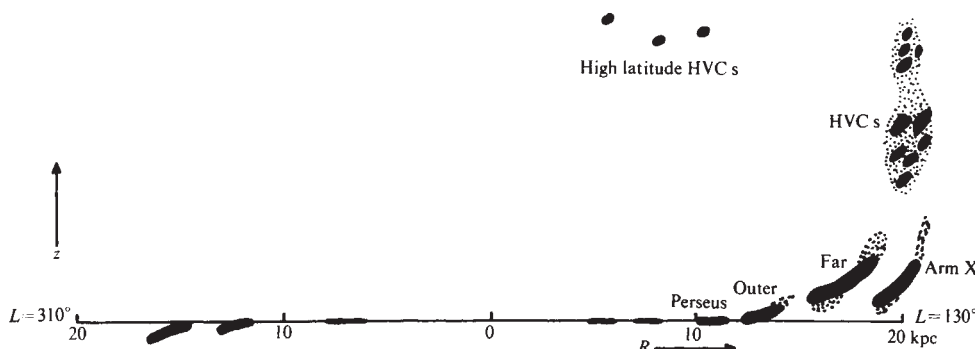


Fig. 3 A sketch of the outer spiral structure of the Galaxy derived from the present observations on the assumption of circular rotation using the Schmidt model¹⁰. The inner spiral structure is shown by the heavy lines; it is taken from Weaver's plot¹¹.

Fig. 4 A sketch of the location of neutral hydrogen in a section through the galactic plane along galactocentric longitudes $L=130^\circ$, 310° . The bands of HVCs have been placed at distances appropriate to their mean velocities and to the spiral arms from which they emerge. The isolated high latitude HVCs cannot be located in this way; they have been schematically placed in accordance with the model proposed in the text.



ness of the emission decreases along the band in moving away from the plane in a trailing sense. The group of positive high velocity clouds at $l \sim 265^\circ$, $b \sim +22^\circ$ has a mean velocity compatible with galactic rotation at a distance of 17 kpc from the galactic centre—a distance comparable with that calculated in the same manner for the negative velocity HVCs at $l \sim 120^\circ$.

Location of HVCs

Two criteria can be used to locate the distance of the HVCs; these may be only partly independent. One is to use the distance of the arm features from which they may emerge and the other is to use the mean velocity of cloud bands and assume circular rotation as for the spiral arms. For the band emerging at $l \sim 100^\circ$, $b \sim +7^\circ$ the results are essentially identical—the band lies at a distance $R=20$ kpc from the centre as a branch of the outer arm. The appearance of the outer arms and the HVC groups as a function of R and height, z , above the plane can be gauged from Fig. 4, where it can be seen that the HVC group just described continues the upward tilt of the galactic plane already evident in the other spiral arms. None of the surveys of

HVCs or outer spiral arms has been extended far enough into southern skies to give information about the tilt beneath the plane which should become evident at $l \gtrsim 270^\circ$.

The steep upward sweep in the outer part of the Galaxy at galactocentric longitude $L=130^\circ$ shown in Fig. 4 is a characteristic of the models so far proposed which explain the tilt in the plane in terms of the tidal effects of the Magellanic Clouds¹⁵⁻¹⁷. At sufficiently great distances from the galactic centre material may break away from the plane altogether—Habing and Visser point out that "at $R=16$ kpc instabilities may occur: mass points may be torn out of the Galaxy"¹⁸. I propose that this phenomenon has indeed occurred, and is demonstrated by the bands of HVCs. Similar effects are clearly seen in interacting galaxies. Furthermore, this leads to a ready explanation of the minority of HVCs which do not fit into the expected range of galactic rotation velocities. They are clouds or groups which have broken away from the outer arms by the supposed tidal interaction of the Magellanic Clouds and are now falling towards the centre of the Galaxy. The observed velocities of these clouds are entirely compatible with such an explanation. This explanation covers the higher latitude HVCs and the negative velocity clouds in the anticentre region.

This model of the HVCs is basically an extension of that originally proposed by Habing in the light of modern data. The HVC pattern is integral with the outermost spiral arms, and, like them, it takes part in the tilt in the galactic plane. It is not necessary, as on the Oort and Dieter models, to propose any origin external to the Galaxy for the high negative velocity gas. All the observed velocities can be explained in terms of a single model.

I thank Miss A. J. Wilson, R. J. Cohen, J. F. Dean and A. Pedlar who assisted in the observing programmes.

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LETTERS TO NATURE

PHYSICAL SCIENCES

Is X Persei an X-ray Source?

It has recently been suggested^{1,2} that the star X Per (HD 24534) may be associated with the X-ray source 2U 0352+30. Other stars identified with X-ray sources are characterized by peculiar spectra with unusual emission lines and by variations in radial velocity which suggests that the sources are binary systems^{3,4}. Because of this we obtained a series of 26 spectra of X Per (including 8 on one night) during the interval December 27, 1971, to March 30, 1972. Dispersions range from 6.5 to 30 Å mm⁻¹, on IIAO, IIAF and IIIAJ emulsions.

X Per has been classified as a peculiar star (Ope). Our spectra show the presence of broad shallow absorption lines of H and He I with emission present in the stronger lines. These and the other lines present suggest that the spectrum is BOe and similar to that of γ Cas. The details of the spectrum are as follows.

First, double emission peaks are seen clearly in H α , H β and H γ . The shortward component is the stronger of the two (by a factor of ~ 3) and can be seen clearly down to $\sim$ H10. Fig. 1 shows some mean profiles and Fig. 2 shows how a blend of absorption and double emission of varying strength can produce all the types of profile observed.

Second, He I lines at $\lambda\lambda 6678$, 5875 and 5015 all show

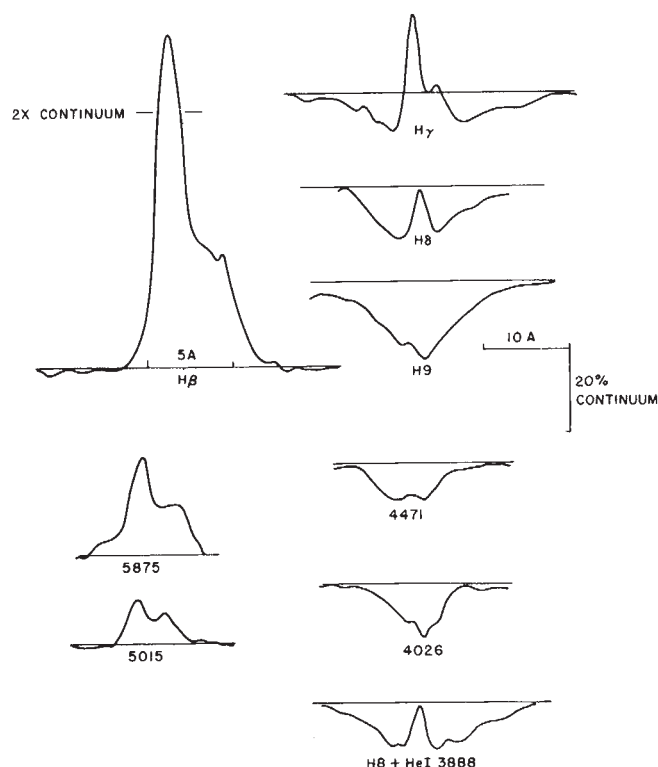


Fig. 1 Mean profiles of Balmer and He I lines. Except for H β , scale indicated at centre, right. Photometric accuracy $\pm 2\%$ of continuum.

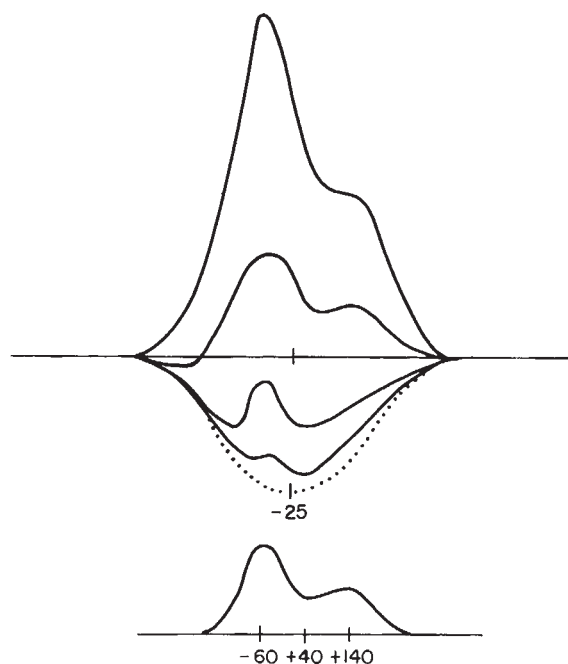


Fig. 2 Schematic diagram of absorption and emission lines. The addition of emission (structured as shown in the lower profile), in varying overall intensity, to the dotted absorption line produces profiles of all the types observed. Numbers are velocities in km s⁻¹.

definite double peaked emission with more nearly equal strengths than the Balmer lines. All other He I lines are asymmetrical, probably because of the presence of emission, as in the Balmer lines. The $\lambda 3888$ line has a strong emission peak (see Table 1), indicating a high density outer envelope.

Third, the two strongest lines apart from H and He I are He II $\lambda 4686$ and Si IV $\lambda 4089$. Interstellar Ca II and Na I are also present, and a number of O II lines can be seen on the IIIAJ plates. Weak N II and N III lines may be present.

We suspect the presence of small variations in several line profiles but only the longward emission component of H β can be said definitely to vary. This variation (up to $\pm 10\%$ of the continuum intensity) seems to occur within minutes and is similar to that found in other Be stars⁵. Like most Be stars, X Per exhibits conspicuous long-term spectrum changes⁶.

Table 1 summarizes the main spectral features. The mean velocities of the absorption and emission systems are indicated in Fig. 2. There is an emission velocity (and peak separation) progression down the Balmer series (from a velocity of -40 km s⁻¹ at H α to -100 km s⁻¹ at H II) and in the He I lines. This effect is known in other Be stars⁷ and is attributable to the geometry of a rotating equatorial ring of rotating gas. The absorption velocities are difficult to measure consistently and show a large scatter (Table 1).

As a whole, these data are not extraordinary for a Be star. But the He I emission strength (especially at $\lambda 3888$) is unusually high, and the velocity difference between the absorption and emission systems is puzzling. (Although the absorption velocities are uncertain, the observed line asymmetries can be produced only by this sort of displacement.) The spectrum is

Table 1 Mean Measurements on Strong Lines in Spectrum of X Per

Line	Short-ward emission peak height (% of continuum)	Peak separation (km s ⁻¹)	V/R (approx.)	V _{emiss} velocity (km s ⁻¹)	R _{emiss} velocity (km s ⁻¹)	Absorption velocity (km s ⁻¹)
H α	500	150	3	-42 $\pm$ 0.6		
H β	270	170	3	-56 $\pm$ 1.1	+102 $\pm$ 5	
H γ	32	180	2.5	-59 $\pm$ 1.4	+129 $\pm$ 5	
H δ	13	250:	3:	-62 $\pm$ 1.5		
H9	3:	270:	1:	-91 $\pm$ 8		-67 $\pm$ 8
H10	3:		1:	-96 $\pm$ 5		-75 $\pm$ 12
H11				-119 $\pm$ 15		
He I						
λ 5875	23	200	2	-51 $\pm$ 3	+138 $\pm$ 2	
λ 4471	5	240:	1.5:	-60 $\pm$ 3	+214:	-111 $\pm$ 7
λ 4026	2:	160:				-43 $\pm$ 4
λ 3819						-32 $\pm$ 6
λ 6678	8	175	1.5	-64 $\pm$ 5	+166 $\pm$ 9	
λ 4921	3	290:		-78:		-36:
λ 4387	2:	230:				+1 $\pm$ 9
λ 4143	2:	270:				-26 $\pm$ 5
λ 3888	12	280:	5:	-49 $\pm$ 2	+176:	
λ 5015	11	190	1.5	-37 $\pm$ 4	+170 $\pm$ 4	
He II						
4686						-22 $\pm$ 8
Si IV						
4089						-13 $\pm$ 15

qualitatively consistent with that expected from a rapidly rotating star surrounded by a rotating, infalling ring⁷.

Available data⁸⁻¹⁰ on the light variations of X Per are poor, but suggest that the star is irregularly (and occasionally rapidly) variable by several tenths of a magnitude. We attempted to fit periods of between two and eight days to the data, with no striking results. Several groups of data suggest a period near 4.5 days but no such period fitted all the data. Blaauw¹¹ noted that the colours of X Per are abnormal, indicating a substantial ultraviolet excess.

Although X Per is similar in several ways to other X-ray source candidates¹²⁻¹⁵, it is not markedly different from a "normal" Be star. We are unable to find evidence for periodic velocity, spectrum or light variations and its final identification as 2U 0352+30 must be left open. We suggest that photoelectric photometry and spectrophotometry of the star may yield more definite information.

We thank R. Giacconi for sending us the catalogue of X-ray sources in advance of publication, and various DAO observers for obtaining spectra.

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Lithospheric Momenta and the Deceleration of the Earth

PROCESSES originating in the interior of the Earth, such as thermal convection and gravitational instability, are not the only ones whereby energy is dissipated at a rate greater than that required to move lithospheric plates. The rate of loss of kinetic energy resulting from the deceleration of the Earth's rotation has been estimated at 7×10^{26} erg yr⁻¹ (ref. 1); this is probably larger than the drag power on the plates.

The site where the forces of deceleration are applied to the rotating Earth is still uncertain. Jeffreys² proposed that the locus of these forces is tidal effects in shallow seas, especially in the Bering Sea. Munk and MacDonald¹ have disputed the estimates of the magnitude of the tidal drag and suggest that only approximately one-quarter of the dissipation can be accounted for and that the problem remains unsolved.

We do not propose to reconsider the question of the mechanism of deceleration. But the application of a brake to the spinning Earth in its outer parts might produce irregular motions of the lithosphere in addition to the primary effect of rigid body deceleration, if the Earth is suitably flawed. If the torque of deceleration corresponds to forces that are applied in regions of the Earth which are irregularly fragmented, then motions of the fragments might result which are not parallel to the forces, because of interactions among the fragments. If the Earth is not fragmented into regions of relative mobility, or if the contacts between fragments are parallel to the forces, or if the forces are distributed as the cosine of the latitude, then no components of motion can be generated in non-azimuthal directions.

Can deceleration be rejected as a mechanism for lithospheric dynamics? This can be determined if the lithosphere has an axial component of angular momentum relative to the lower mantle, in a sense opposite to that of the ordinary spin angular momentum. The analysis requires the existence of an inertial frame of reference fixed in the lower mantle; unfortunately, we know only the relative motions among the lithospheric plates and not the motions in an inertial frame.

This difficulty may be overcome because of the approximate symmetry of the Antarctic plate relative to the axis of rotation. Not only is the Antarctic plate located astride the geographic pole, but it is surrounded by oceanic rifts, that is, loci of spreading, at almost its entire circumference. Thus, unless the Antarctic plate has a relatively high velocity past the pole of rotation³, it seems that it has a symmetry which, for our purposes, provides an inertial frame.

We have recalculated the relative velocities of ten lithospheric plates, instead of the six plates considered by LePichon⁴, using more recent information. In the east Pacific, the Cocos and Nazca plates are added; a Philippine plate is defined in the west Pacific; the Eurasian plate is split into the European and Chinese plates; and the boundaries between the American and Eurasian plates are redrawn in the Siberian region, to agree with the seismic evidence of Tarr⁵.

Because ten plates are used, nine independent angular velocities are needed to determine the motions completely. The pole positions for African-American, Pacific-Antarctic, American-Pacific, Indian-African and European plates are taken from LePichon⁴. We recalculated angular velocities from the spreading rate data with results close to those of LePichon. Some of the data for the East Pacific Rise are now used for the Cocos-Pacific and Nazca-Pacific interactions; this has only a small effect on the Pacific-Antarctic rate. For the American-Pacific motion, we used Morgan's rate⁶, based on movement of the San Andreas Fault. This leads to minor differences from LePichon's values in the poles derived.

For the east Pacific, the Cocos-Pacific pole and rate were taken from Larson and Chase⁷. The poles for the Nazca plate were selected to fit the slip direction on the Easter Island fracture zone plus the spreading rates for the East Pacific and

Chile Rises. For a small plate, the angular velocity vector is poorly determined, even though the linear velocity may be well known.

We define the European-American boundary along the mid-Arctic Ridge to Sakhalin; the American-Chinese boundary runs from Sakhalin to Hokkaido; and the European-Chinese boundary runs west from Sakhalin to Lake Baikal and then south to the Himalaya-Panir. Poles were chosen to give compression along the Java trench, opening at Lake Baikal, and small strike-slip motion from Lake Baikal to Sakhalin. The Philippine-Pacific pole is taken from Katsumata and Sykes⁸ with a rate chosen consistent with the assumption that the deepest earthquakes in the Mariana seismic zone correspond to a subduction of lithospheric matter accomplished in 10 m.y. (ref. 9).

Table 1 Angular Velocities of Spreading

Plate pair	Latitude	Longitude	Velocity (cm yr ⁻¹) *
Eu-Am	78.0° N	102.0° E	3.2
Af-Am	58.0° N	37.0° W	4.0
Af-In	25.9° S	159.1° W	4.8
Am-Pa	53.0° N	47.0° W	8.0
Ch-In	18.0° S	140.0° W	8.2
Pa-Ph	7.0° N	142.0° E	7.6
Pa-Co	40.0° S	70.0° E	21.8
Pa-An	70.0° S	118.0° E	12.2
Na-An	9.0° N	98.0° W	6.4
Eu-Af	5.6° S	133.5° E	2.7
Eu-Ch	1.8° N	88.9° E	5.4
Eu-In	21.9° S	176.3° E	6.3
Af-An	27.6° S	20.3° W	3.6
Am-Ch	31.9° S	87.1° E	5.6
Am-Co	26.8° S	53.5° E	16.9
Am-Na	41.8° S	71.0° E	9.1
Am-An	75.3° S	7.0° E	5.2
Ch-Pa	46.7° N	69.8° W	12.8
Ch-Ph	65.7° N	128.7° W	11.3
Pa-In	51.1° S	160.2° E	15.3
Pa-Na	51.3° S	96.2° E	16.0
In-An	3.4° N	3.5° E	7.1
Co-Na	9.9° N	139.7° W	9.0
Eu-Pa	65.9° N	42.4° W	10.4
Eu-Ph	72.4° N	147.7° E	11.0
Eu-Co	16.1° S	55.4° E	16.2
Eu-Na	21.8° S	73.7° E	8.0
Eu-An	53.5° S	34.4° E	2.4
Af-Ch	6.2° N	60.9° E	4.0
Af-Pa	54.8° N	44.0° W	12.0
Af-Ph	84.8° N	171.0° W	10.8
Af-Co	15.5° S	45.6° E	15.8
Af-Na	22.6° S	52.9° E	7.0
Am-Ph	68.5° N	157.1° E	7.9
Am-In	43.8° S	177.3° W	7.9
Ch-Co	22.1° S	40.3° E	12.4
Ch-Na	50.3° S	40.5° E	4.0
Ch-An	24.2° S	76.7° W	5.2
Ph-In	75.3° S	155.6° W	13.2
Ph-Co	42.9° S	43.5° E	21.9
Ph-Na	61.8° S	47.4° E	15.2
Ph-An	71.7° S	13.5° W	13.1
In-Co	6.3° S	40.2° E	19.4
In-Na	3.2° S	40.1° E	10.4
Co-An	10.2° N	122.6° W	14.5

* Note that in ref. 4 separation is given as ° yr⁻¹.

The poles of rotation for the relative motions are given in Table 1 for all 45 combinations of plate pairs; the velocities of relative motion are those at the equator corresponding to the pole of spreading, whether this equator intersects the contact between the two plates or not. The plate pairs in the upper part of Table 1 represent the independent terms in the solution of the simultaneous equations; the velocities for the remaining pairs are derived from these primary values. The plate pairs at the bottom of Table 1 do not have a common contact; relative velocities can still be obtained in the usual way. The pole is that for the motion of the first plate listed, as

though the second listed were stationary, with the sign of angular velocity according to the right hand rule.

We have calculated the angular momentum of all the plates relative to the Antarctic plate, projected on the cartesian coordinates determined by the north pole (0°, 0°) and (0°, 90° E), in units of HSL cm yr⁻¹. (The HSL, or hourly semi-lune, is the area of the Earth's surface described by the spherical triangle consisting of two lines of longitude 15° apart and the equator. The total area of the Earth's surface is 48 HSL.) In these units, the thickness of all lithospheric plates, their densities and their distances from the centre of the Earth each have unit values. The surface area of each of the lithospheric plates is given in Table 2. The angular momentum of all the plates is (15.3, 15.6, -73.0).

Table 2 Momenta of Lithospheric Plates

Plate	Area (HSL)	Angular momentum relative to Antarctic (HSL cm yr ⁻¹)			Momentum toward equator (HSL cm yr ⁻¹)
		x	y	z	
Europe	3.5	2.4	1.8	-2.0	-0.2
Africa	7.2	4.0	-4.8	-5.1	1.9
America	9.9	3.3	-0.5	-13.9	1.2
China	2.2	-0.3	-0.8	-0.3	0.4
Pacific	10.45	-9.8	17.7	-47.8	-1.5
Philippine	0.7	-0.1	1.4	-3.8	-0.8
Indian	6.6	17.1	2.0	-2.6	6.8
Cocos	0.3	-0.8	0.1	0.1	-0.8
Nazca	1.65	-0.5	-1.2	2.3	0.4
Antarctica	5.5	0	0	0	0
Total	48.0	15.3	15.6	-73.0	7.4

The dominant term is clearly that pointing along the axis of rotation. The sign is appropriate to that of deceleration of the Earth, that is, there is a westward drift of the lithospheric plates relative to Antarctica. More than one-half of the total is contributed by the motion of the Pacific plate relative to Antarctica. The mean axial component of velocity of drift is 2.3 cm yr⁻¹ (the angular momentum of a rigid thin spherical shell of radius a is $\frac{2}{3} Ma^2\omega$, with M the mass and ω the angular velocity).

Bostrom⁹ has proposed that the westerly drift of the lithosphere is a result of tidal drag. It can be shown¹ that this effect is too small to explain the powers of earthquake generation of lithospheric drag in the low velocity channel. The amount of tidal energy liberated per year in a layer of thickness h at the surface is

$$(\frac{1}{2}\mu\epsilon^2)(4\pi a^2h)(730)/Q$$

The first term is the shear strain energy density with μ the shear modulus and ϵ the shear strain; the second term is the volume of the layer with a the radius of the Earth; the factor 730 is the number of semi-diurnal cycles per year and Q is the specific dissipation factor for the surface layer. If $h=600$ km, $a\epsilon=30$ cm, $\mu=8\times 10^{11}$ dy cm⁻² and Q is taken optimistically to be as low as 80 for the low-velocity channel, then about 3×10^{24} erg yr⁻¹ are derived from this source, much less than the energy required from an important source. A supposed correlation of earthquake activity with the times of lunar perigee and syzygy¹⁰ reported in corroboration by Bostrom⁹, has been shown to be completely spurious¹¹.

We conclude that deceleration cannot be disregarded as a possible cause of driving lithospheric plate motions. The cause of the deceleration remains unknown.

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Rifting in the Kalahari?

A RECENT study of published earthquake epicentres for Botswana in central southern Africa reveals a high level of seismic activity over the vast areas of the country where the solid geology is almost totally obscured by Tertiary to Recent Kalahari Sands. Between September 1965 and August 1971 the seismograph network of the Rhodesia Meteorological Services located fifty epicentres within Botswana¹. Most of these events were of Richter magnitude 3 or smaller, but epicentres for one magnitude 5 and four magnitude 4 events were among those published. By using the data from the local seismograph network many smaller events become available in addition to those used by Fairhead and Girdler² from the Worldwide Standardized Seismograph Network (WWSSN) data.

The Rhodesia network consisted of five Willmore seismographs sited in Rhodesia, Malawi and Zambia. The data from these stations were supplemented by arrival times at stations in the South Africa network, particularly Windhoek and Pretoria, when available. An accuracy of about ± 50 km should have been obtained for the epicentres published, the distribution of recording stations with respect to Botswana being far from ideal.

The epicentres form two distinct spatial populations. First, a dense cluster of twenty-eight events over the swamps of the Okavango Delta in the Northwest District (Ngamiland); second, twenty events in a broadly scattered belt trending north-east across the Central Kalahari to which I have fitted a linear regression line (Fig. 1).

Both the Central Kalahari Basin and the Okavango Delta are characterized by extremely low topographic relief, the continental plateau standing about 1,000 m above sea level. Geological data from the eastern exposed areas and shallow drilling for water elsewhere reveal that Karroo sediments and volcanics underlie much of the Kalahari³, but drilling up to 170 m (500 feet) in the Okavango has found only unconsolidated sediments presumably of recent origin. The ubiquitous sand cover and problems of accessibility militate against conventional geological and geophysical exploration of the country. The distribution of earthquakes is therefore an important new piece of information.

Seismicity in the Okavango was first recognized in 1952 when a series of two magnitude 6 and twenty-one magnitude events was located there by the early South African seismographs^{4,5}. These events are substantiated by records of damage to buildings at Maun (Geological Survey and Mines Department). Several major linear features, most about 100 km in length, have long been recognized bounding the Okavango (Fig. 1), and in the absence of direct geological evidence they can only be interpreted as major faults. Even so, they have attracted little attention from previous authors on rifting in Africa^{2,6,7}. On the ground the faults have little or no topographic expression, though it is possible that sedimentation has kept pace with subsidence, in view of the abundant provenance of the Okavango headwaters. To elucidate the geological structures of this remote area this department has established 1,500 gravity stations over some 80,000 km² in and around the delta since 1970, and the results of the survey are

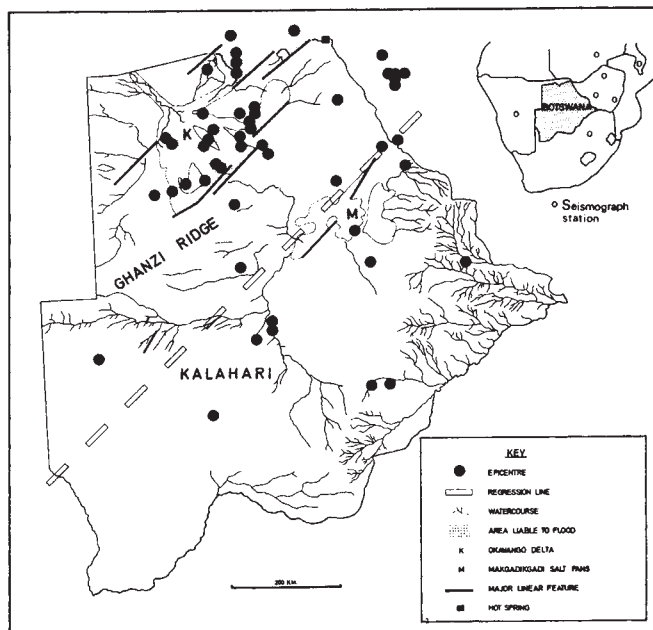


Fig. 1 Earthquake epicentres in Botswana, September 1965 to August 1971.

in preparation. Unfortunately since the introduction of the WWSSN no seismic event in the Okavango has been large enough to enable a fault plane solution to be made (J. D. Fairhead, personal communication). Such a demonstration of normal faulting along a north-east strike would give much added support to the rifting hypothesis.

The Okavango is separated from the Central Kalahari Basin by the Ghanzi Ridge, a broad aseismic upwarp of little explored Palaeozoic sediments isoclinally folded along a north-east strike. Similar or related rocks of Damara age presumably extend at depth both to the north below the Okavango Delta, and to the south below at least part of the Kalahari, but the extent in area of Damarides within Botswana is uncertain.

The linear regression through the twenty Kalahari epicentres (the "Kalahari Seismicity Axis") closely parallels the linear features of the Makgadikgadi Salt Pans, the linear features of the Okavango Delta and the Ghanzi Ridge (Fig. 1). Seismicity seems to reflect regional subsidence because the lowest lying area of the Kalahari Basin (the Makgadikgadi) falls astride the axis and the "fossil" drainage system of the Kalahari has a marked north-east strike bias. Kimberlite pipes of the recently discovered Orapa diamond fields south-west of the Makgadikgadi penetrate Karroo Basalt and provide direct geological evidence of post-Karroo volcanism close to the axis of present day seismicity.

Does the Kalahari seismicity represent the closing stages of more active rifting akin to that now observed in the Okavango? The Kalahari seismic axis is a well aligned extension to the south of the Zambesi Valley of Zambia's Karroo-filled Luangwa Rift⁶. It is possible that migratory rift-faulting has also been the controlling force of Karroo sedimentation and volcanism in the Kalahari. The extent to which the distribution of the Karroo in the Kalahari was controlled by structures in the earlier Damara formations remains uncertain, as does the question of whether these observations can be reconciled, any more or less than the better defined rifts of East Africa, with plate tectonics theory.

The north-easterly grain of the Kalahari is certainly a long lived one, probably predating the Atlantic Ocean if active during the Karroo, but the total accretion of new crustal material across this axis seems even smaller than in the East African Rifts, so the spreading rate must be minimal. Because of its apparent age, any southern extension of the African Rift System into the Kalahari is perhaps not an even earlier phase of continental break-up² but the vestige of a mode of break-up

which never really got started, now conserved in the guise of a sub-plate boundary.

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Possible Impact Structure in India

THE Survey of India topographical sheet 54C, Sawai Madhopur, scale 4 miles to 1 inch (first published in 1908), shows a latitude 25° 20' N, longitude 76° 37' 30" E, an isolated hill of annular shape, forming an almost complete circle approximately 3 km in external diameter. This feature lies in eastern Rajasthan almost on the boundary with Madhya Pradesh, approximately 350 km SSW of New Delhi and approximately 13 km east of the small town of Mangrol¹. It is east of the Parbati river on the interfluvium between it and a right bank tributary, the Kul. The country there is almost flat, and although the region lies within the general outcrop of the upper Precambrian Vindhyan System, the bedrock is almost entirely obscured by a wide belt of alluvium. Where Vindhyan rocks are exposed in the vicinity, as in the bed of the Parbati river 6 km east of Mangrol, they are flat-lying and undisturbed.

Because the hill is annular it cannot be a residual mesa of Vindhyan rocks higher in the sequence, or one of Deccan Trap, the main outcrop of which lies about 50 km to the south. The feature seems likely to be of local and anomalous tectonic origin, possibly diapiric, or the result of some kind of impact. The area has never been geologically mapped except for a general reconnaissance by Mallet², who made no reference to the feature, in 1869; if seen from a distance this could well be mistaken for either a Vindhyan mesa or a Deccan Trap residual of characteristic shape.

In April 1970 I visited the locality with V. D. Mahajan of the Geological Survey of India. The feature consists of a ring of Vindhyan-type red quartzitic sandstone, rising approximately 200 m above the plain. The low central area is little higher than the plain. The ring is breached behind the village of Ramgarh,



Fig. 1 View, from 10 km west, of possible impact structure in India.

which lies at the foot of the hill on its south-west side, and after which the feature is named. Examination of the exposures in the narrow breach showed massive red quartzitic sandstones with dips to the south-west which increase steeply towards the inner wall, which is generally rather steeper than the outer wall. Locally, brecciated rock occurs without any specific indication of faulting. A specimen found in colluvium near the centre of the feature seems shatter-coned.

The feature is illustrated cartographically and air-photographically in the *School Atlas*³ of the Indian Ministry of Education as an example of a "hill feature". The air photograph shows clearly that the feature is not exactly circular but almost rectangular, with rounded corners. As pointed out by D. J. Milton (personal communication) of the US Geological Survey, Meteor Crater, Arizona, has a similar shape.

The feature seems likely to be some kind of impact structure. Further work by the Geological Survey of India is in progress.

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The Internal Surge in Loch Ness

THE large temperature changes which occur at depth in Loch Ness are caused by an internal seiche or standing wave^{1,2}, which develops a pronounced front or surge like a tidal bore and is sometimes followed by a periodic oscillation of the isotherms. The surge is produced by the stress of the wind on the water surface^{2,3}, which causes a flow of near-surface water to one end of the Loch and a depression of the thermocline at that end. When the wind falls, the tendency of the thermocline to recover a level position initiates the surge, which is often over 10 m in height. The following wave train, when it occurs, is of comparable height and contains waves about 1 km long. No effects are seen, or are expected to be seen, at the surface. In calm weather the surge progresses up and down the Loch for more than a week, during which time it travels more than 200 km (refs. 2 and 4).

During the summer of 1971 we examined the relation between the thermal microstructure and the internal surge. Records obtained from fixed moorings clearly show the passage of the surge and conclusively demonstrate its progressive nature. The positions of the moorings are shown in Fig. 1. We present here some of the measurements of a typical surge which were obtained between 1800 h on October 2 and 2400 h on October 3.

On the evening of September 30, a strong SW wind blew up and continued until late afternoon of October 1. The wind probably exceeded 13 m s⁻¹ in mid-loch at midnight on September 30 and speeds of 10.3 m s⁻¹ were measured in mid-loch at 1430 h on October 1; it was this wind which generated the surge which returned from the SW to the position of our moorings during the night of October 2. There was a SW breeze on October 2 but this was not strong enough, or of sufficient duration, to have any effect on the thermocline, and otherwise the weather remained calm until the afternoon of October 3. The early morning of October 3 was a glassy calm with thick mist hanging low over the Loch, but this lifted at about 1000 h. The wind rose at midday and blew strongly from the NE with speed exceeding 9 m s⁻¹ during the afternoon, causing steep breaking waves and foam streaks on the Loch surface. The wind fell during the evening and the Loch was fairly calm by 2300 h.

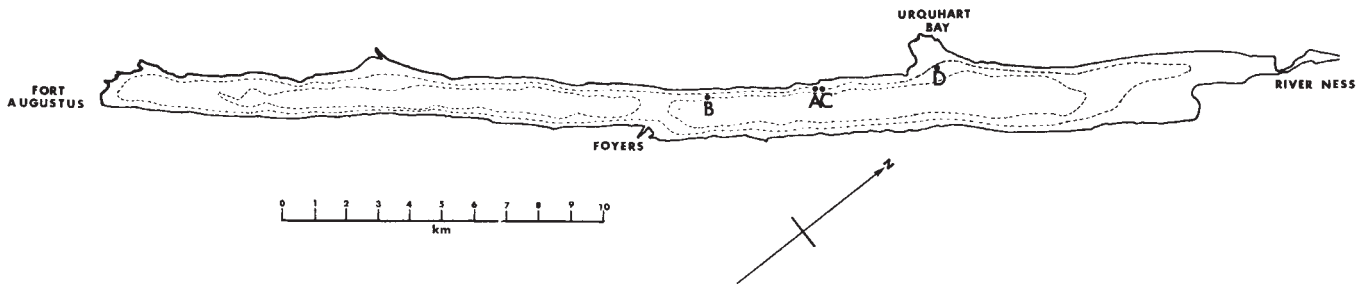


Fig. 1 Loch Ness. The 300 and 600 foot depth contours are shown by dashed lines. A is a winch driven probe operated from land, used to obtain temperature profiles (see Fig. 3). It was particularly useful in rough conditions when mid-loch operations became hazardous. Mooring B (water depth 184 m) carries two Bergen current meters at 11 m and 48 m below the surface with buoyancy at 1 m and a surface dhan buoy. Mooring C (103 m) carries a single Bergen current meter at 40 m with buoyancy at 20 m. These current meters recorded temperature and the current magnitude and direction every 15 min. Mooring D (137 m) is a chain of ten thermistors at various selected depths between 23 m and 88 m. This was connected by a cable to land and the temperatures were recorded continuously by a speedomax chart recorder and every 2 min on paper tape. Waves with periods down to 10 min were observed on the chart recorder.

The surge is best seen in the records of temperature (Fig. 2). The top current meter (11 m) at mooring B was in the upper mixed layer and showed no significant change. The temperatures recorded by the other thermistors on mooring D moved in phase with those shown in Fig. 2 during the arrival of the surge and in the following wave train, showing that they belong to a first order internal mode. The surge propagated in an undular form to the NE and was later reflected to the SW and passed the moorings in turn as a simple change in level. The increase in the temperatures as the surge passed shows that the isotherms were lowered and that the surge was a wave of depression in accordance with theoretical predictions². Fig. 3 shows the change in the temperature structure and the isotherms as the surge passed A. The change in level of the isotherms was about 12 m. The mean speed of propagation of the surge found from the arrival times at the moorings was 36.8 cm s^{-1} .

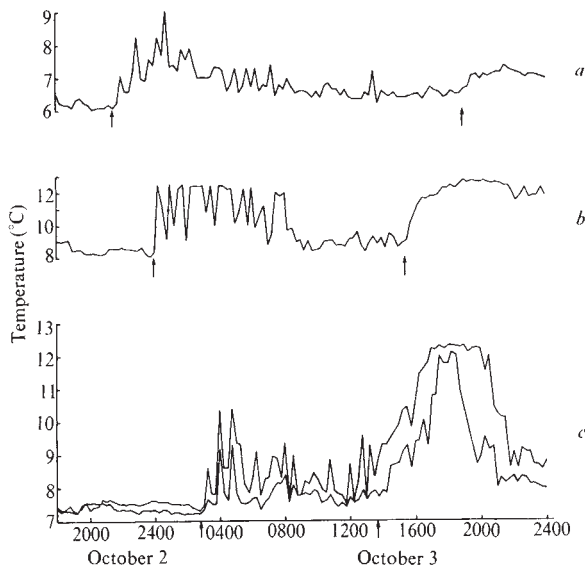


Fig. 2 The temperatures at: (a) 48 m depth, mooring B; (b) 40 m depth at mooring C; (c) 43 m and 48 m depth, mooring D. The arrival of the surge at each mooring is marked by vertical arrows.

The currents and their directions at the three current meters are shown in Fig. 4. The first arrival of the surge at mooring B (11 m) was clearly marked by an oscillation and shift in current direction from SW to NE. This near surface flow following the surge to the NE was also found at point C (40 m), but the lower current meter at B (48 m) showed variable directions after the first arrival of the surge and eventually (at about 1000 h) the current fell slack and then set to the SW as the isotherms rose; the meter was below the level of zero mean

current in the first mode internal wave. The return of the surge from the NE was preceded at this mooring by the onset of the NE wind. The apparent increase in current recorded by both meters at point B after 1200 h was the result of the motion of the mooring induced by surface wave action. The direction of the current at point C (40 m) changed from NE to SW at 1700 h as the 11.9°C isotherm fell past the level of the current meter in the lee of the surge. The change in the direction of the current at B (11 m) was well before the arrival of the surge and appears to be the result of a wind-stress induced drift to the SW, but the change in direction at B (48 m) from SW to NE at 1900 h, when the surge arrived, shows that this current meter was below the level of zero mean current in the thermocline.

The small changes in direction and magnitude of the current at C (40 m) appear to correspond to induced water motions up and down the sloping sides of the Loch as the undular surge passed. The waves which followed the surge had a period of about 40 min; this did not change when the surge reached D where the Loch is wider at the 40 m level, and it seems very unlikely that these waves were caused by a transverse oscillation; we interpret them as being waves propagating with the surge front with a wavelength of about 1 km and similar to waves observed in the laboratory². The surge probably

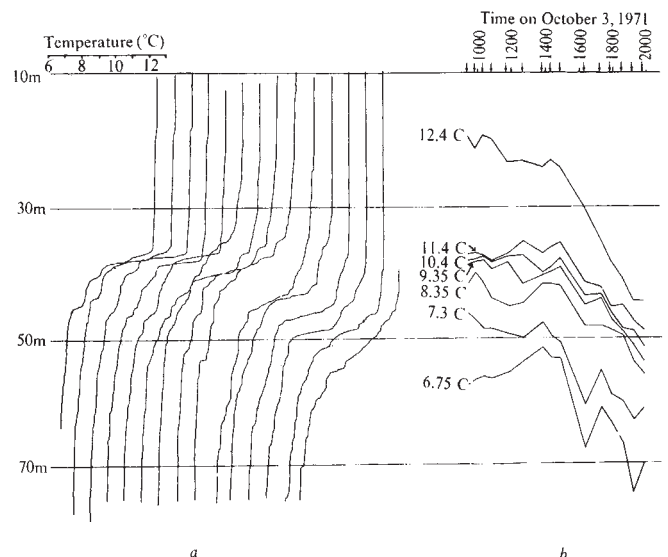


Fig. 3 (a) Temperature profiles at A between 10 m and 75 m on October 3, 1971. The temperature scale is for profile (1) and the other profiles have been moved successively 1°C to the right. (b) The times at which the profiles were made, indicated by vertical arrows, and the variation in the isotherm levels during the period of observation.

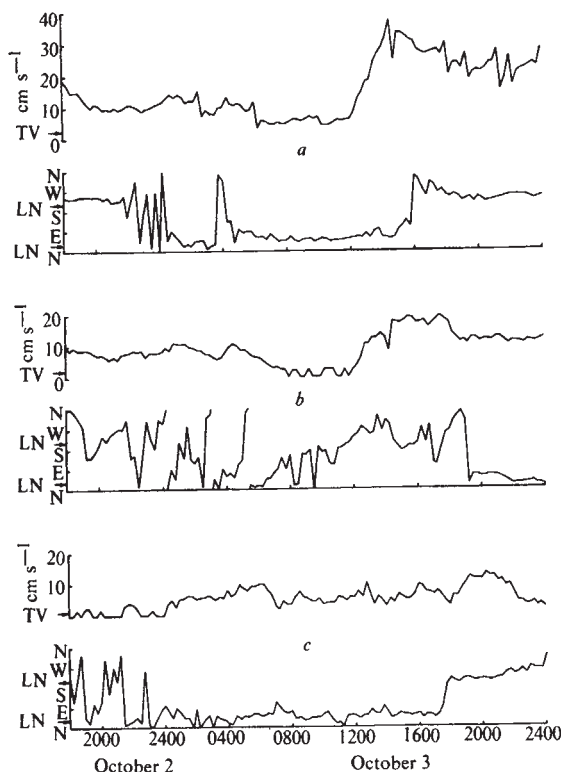


Fig. 4 The currents and their directions at: (a) 11 m depth, mooring B; (b) 48 m depth, mooring B; (c) 40 m depth at mooring C. The current magnitudes at mooring B are seriously affected by the effect of mooring motions after 1200 h on October 3. The threshold values of the current meters (TV) and the orientation of Loch Ness (LN) are shown on the current magnitude and direction axes respectively.

propagates as a Kelvin wave with the largest amplitude on the right facing the direction of advance, the SE side as the surge travels to the NE. The reflexion of the surge at the NE end of the Loch seems to dissipate the accompanying wave train, presumably as the result of wave breaking on the slope (about 1 in 50) and refraction at the irregularly shaped end at 40 m depth. The speed of the surge will be close to that of a long internal wave in a non-rotating system, and can be estimated from a three layer model with constant density in the upper and lower layers and an exponential increase of density with depth in the central thermocline layer. Taking 40 m, 100 m and 10 m as the respective thicknesses of the layers, and the density difference corresponding to a temperature change from top to bottom of 12.5°C to 6.5°C (the Loch is fresh water), we find that the speed of the surge is 36.0 cm s^{-1} which agrees well with the observed speed. We expect some increase in this estimate when the effects of finite amplitude are included.

The stability of the water column to shear, or Kelvin-Helmholtz instability, during the passage of the surge is determined by the gradient Richardson number, Ri , and for a long first mode internal wave this may be estimated⁵ from a knowledge of the wave amplitude a , the wave speed c , and the local Brunt-Väisälä frequency, N ; $Ri = c^2/N^2a^2$. The largest value of N observed in the fine structure of the thermocline was about $4.5 \times 10^{-2}\text{ s}^{-1}$. Taking typical values $a = 8\text{ m}$, $c = 37\text{ cm s}^{-1}$, the smallest Ri attained during the passage of the surge is approximately 1. This is greater than that necessary to initiate instability in a steady two-dimensional shear flow, and it therefore seems hardly unlikely that much of the energy of the surge is dissipated in turbulence arising directly from a "self induced" shear instability in mid-loch, or that the thermal microstructure is directly generated from such a process (the water motions directly induced by the surge at the sides and ends of the Loch are much less likely to remain stable). Only a small reduction in Ri is required before Kelvin-Helmholtz instability

can occur in the high temperature gradient regions, so the amplitude of the surge is probably limited by Kelvin-Helmholtz instability, when the shear induced by the surge is added to the mean shear in the existing internal wave background noise in the Loch. An understanding of the spectrum of the internal waves is thus a necessary prerequisite for the determination of the stability of the surge. The temperature fluctuations recorded at the thermistor chain, D , will provide information about the spectrum and its variation in the presence of the known thermal microstructure.

Similar surges may be expected in other lakes or fjords, and near sea coasts after the fall of an on-shore or off-shore wind.

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Estimate of the World Budget of Fallout Silver Nuclides

THE increasing use of silver iodide in cloud-seeding has stimulated interest in the behaviour of silver in the marine environment. In view of this, more information about the ecological fate of fallout silver¹⁻⁴ is needed. Recently an estimate was made⁵ for the minimum northern hemisphere budget of ^{110m}Ag created during nuclear weapons testing in 1961-62. This estimate was made from the concentration of ^{110m}Ag measured in the livers of nine tuna fish caught in the North Pacific in 1965, together with the following assumptions: a concentration factor for silver in the livers over seawater of 500,000; a volume of the top 100 m of the North Pacific of $7.1 \times 10^{18}\text{ l}$; and that the total area of the northern hemisphere is $2.55 \times 10^8\text{ km}^2$. The estimated budget of ^{110m}Ag over the surface layers of the northern hemisphere at the end of 1962 was computed to be at least 100,000 Ci.

It can be shown, however, that an entirely independent approximation of the ^{110m}Ag budget can be made without using any of these assumptions. This can be done from the ratios of reported concentrations of fallout ^{90}Sr and ^{110m}Ag in sixty air filters⁶ collected at high altitudes over the northern

Table 1 Trends of the $^{90}\text{Sr}/^{110m}\text{Ag}$ Ratios reported for High Altitude Air Filters from the Northern Hemisphere, June to December 1963 (see Ref. 6)

Date	No. of filters per sample	Average $^{90}\text{Sr}/^{110m}\text{Ag}$
June	5	88
July	10	151
August	12	151
September	9	192
October	7	140
November	9	172
December	8	211

Average corrected to mid-September ~ 160 .

BIOLOGICAL SCIENCES

Electron Microscopic Studies on Substrate Specificity of T4 Excision Repair Endonuclease

FOLLOWING infection of a suitable host by bacteriophage T4, a phage-specific endonuclease that makes single-strand breaks in ultraviolet-irradiated DNA^{1,2} is produced. This enzyme is presumably involved in pyrimidine dimer excision, since a mutant phage T4v₁, which does not produce detectable enzyme, is abnormally sensitive to ultraviolet light and fails to excise dimers from its DNA³. This endonuclease has been extensively purified⁴. Previous *in vitro* studies utilizing sedimentation velocity of DNA in sucrose density gradients showed ultraviolet dose-dependent reduction in sedimentation velocity of ultraviolet-irradiated T4 and T7 DNA following incubation with the purified enzyme. The sedimentation velocity of unirradiated DNA was not detectably reduced. Furthermore, the average number of enzymatically-induced nicks per molecule correlated well with the calculated number of dimers per molecule⁴. These studies suggested that the only substrate sites in ultraviolet-irradiated DNA were pyrimidine dimers. Unirradiated DNA was unaffected by the enzyme and it was concluded that such DNA either does not contain areas of distortion in the secondary structure such as might be produced by pyrimidine dimers, or if such distortions are present they are not recognized as substrate sites by the endonuclease.

As these conclusions were based on the results of sucrose density gradient centrifugation, the presence of a limited number of nicks in a small percentage of a population of unirradiated DNA molecules could go undetected. In addition, the stoichiometric correlation between endonucleolytic incisions and pyrimidine dimers was based on a calculation of the dimer content of DNA from measurements of the percentage thymine dimer in radioactively-labelled *E. coli* DNA⁵. At low doses of ultraviolet irradiation, the thymine dimer component constitutes a very small fraction of the total labelled thymine (0.06% in *E. coli* DNA at 100 ergs/mm²; ref. 5), so that an accurate determination of the dimer content of DNA irradiated at low doses is difficult and is usually obtained by direct extrapolation from measurements made at much higher doses.

We describe here the use of a technique which provides extraordinary sensitivity for the detection of phosphodiester bond scissions in DNA and also allows the determination of the pyrimidine dimer content of ultraviolet-irradiated DNA by a totally independent method which does not require radioactive labelling. The procedure involves the differentiation of supercoiled circular SV40 DNA from relaxed circular DNA, a task that is readily accomplished by electron microscopic examination. As the change from the supercoiled to the relaxed state is dependent on a single phosphodiester bond break⁶, this technique provides the required sensitivity for examination of the substrate specificity of the T4 excision repair endonuclease. Furthermore, by measuring the relative frequency of supercoiled and relaxed circles following enzymatic incubation of SV40 DNA that was ultraviolet-irradiated at very low doses, it was possible to demonstrate a Poisson distribution of dimers, thereby allowing a direct determination of the average dimer content of the DNA.

SV40 DNA ultraviolet-irradiated at 300 ergs/mm² without enzyme addition showed no evidence of radiation-induced phosphodiester bond breakage. Incubation of unirradiated SV40 DNA with endonuclease did not show any reduction in the fraction of form I molecules. This experiment was performed with and without added MgCl₂ with the same result. Purified T4 excision repair endonuclease clearly does not nick unirradiated DNA. On the other hand, ultraviolet-irradiated DNA is attacked by the endonuclease resulting in

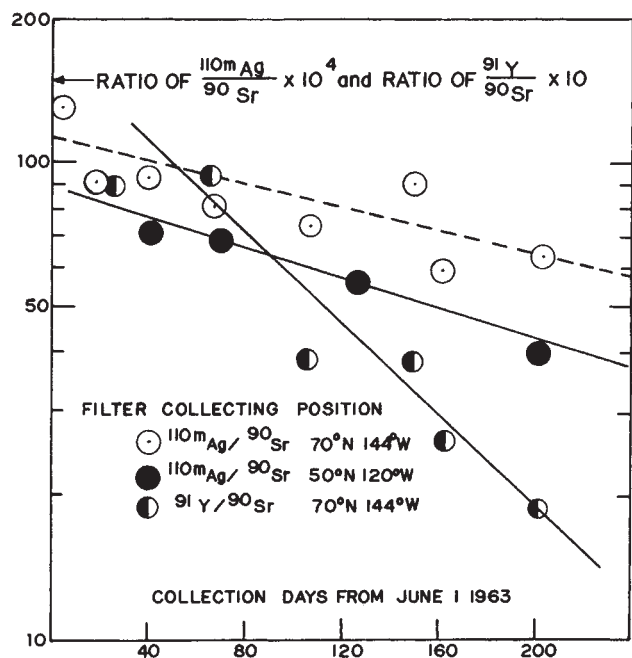


Fig. 1 $^{110m}\text{Ag}/^{90}\text{Sr}$ and $^{91}\text{Y}/^{90}\text{Sr}$ activity ratios based on measurements by HASL in high altitude air filters.

hemisphere from July to December 1963. Since ^{110m}Ag has sometimes been mistaken for ^{137}Cs in complicated γ spectra, we sought to identify silver by its decay rate. Fig. 1 shows the $^{110m}\text{Ag}/^{90}\text{Sr}$ activity ratios found in filters collected monthly at two different locations. For comparison, $^{91}\text{Y}/^{90}\text{Sr}$ ratios in the same filters at one location are also plotted. The observed half-lives of 220 ± 40 days for ^{110m}Ag and 64 ± 15 days for ^{91}Y are close to the actual values of 253 and 57 days respectively. Table 1 lists the average monthly $^{90}\text{Sr}/^{110m}\text{Ag}$ ratios for all locations.

The average value of $^{90}\text{Sr}/^{110m}\text{Ag}$ when extrapolated back to December 1962 is 80. With this ratio and the reported⁷ 1961–62 world budget of ^{90}Sr of about 10.1 MCi, the world budget of ^{110m}Ag at the end of 1962 is $\sim 126,000$ Ci. This is in excellent agreement with the minimum estimate of 100,000 Ci made by Grismore, an estimate that depended largely on the assumed mean behaviour of a silver nuclide in an organ of an oceanic fish.

This study is continuing at Scripps toward determining the world budget of the long-lived (127 yr) ^{108m}Ag . We have recently measured ^{108m}Ag in more than fifty marine organisms. It seems that the activity ratio of $^{90}\text{Sr}/^{108m}\text{Ag}$ may be roughly 16,000. More knowledge about the ratio of $^{90}\text{Sr}/^{108m}\text{Ag}$ would be of help in predicting how this silver nuclide tag can be used to study natural silver in the oceans.

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² Folsom, T. R., Grismore, R., and Young, D. R., *Nature*, **227**, 941 (1970).

³ Beasley, T. M., and Held, E. E., *Nature*, **230**, 450 (1971).

⁴ Jenkins, C. E., and Cooper, J. A., *Pacific Northwest Laboratory Annual Report for 1970*, BNWL-1551, Part 2, UC-48, 6 (1971).

⁵ Grismore, R., Folsom, T. R., Hodge, V. F., and Young, D. R., *Trans. New York Acad. Sci.* (in the press).

⁶ Feely, H. W., Friend, J. P., Lagomarsino, R. J., Bogen, D. C., Biscaye, P. E., and Hardaway, J. E., *US Atomic Energy Comm. Health and Safety Lab. Rep.*, HASL-168, 197 (1966).

⁷ Federal Radiation Council, *Report No. 4—Estimates and Evaluation of Fallout in the United States from Nuclear Weapons Testing Conducted through 1962*, 5 (US Government Printing Office, Washington DC, 1963).

the conversion of form I to form II molecules (Fig. 1). No linear DNA molecules were ever observed, confirming the observation that the endonuclease makes only single-strand breaks^{1,4}. Fig. 2 shows the result of a series of incubations of enzyme with SV40 DNA ultraviolet-irradiated at doses between 0–200 ergs/mm². After complete reaction the fraction of form I molecules follows a Poisson distribution as a function of the ultraviolet dose. According to this distribution, 37% of the molecules are in the form I state when there has been an average of 1 endonucleolytic incision per molecule. If every endonucleolytic incision occurred at a pyrimidine dimer, the ultraviolet dose at which 37% of the molecules are in the form I state should have produced an average of one dimer per molecule. The curve in Fig. 2 indicates that 37% of the enzyme-treated molecules retain the form I configuration at an ultraviolet exposure time corresponding to 21.6 s (172.8 ergs/mm²/s). Thus 172.8 ergs/mm²/s produces an average of one pyrimidine dimer/3.2 × 10⁶ molecular weight of DNA. Assuming a molecular weight of 2.8 × 10⁹ for *E. coli* DNA,

1 erg/mm² would produce an average of $\frac{2.8 \times 10^9}{3.2 \times 10^6} \times \frac{1}{172.8}$ or

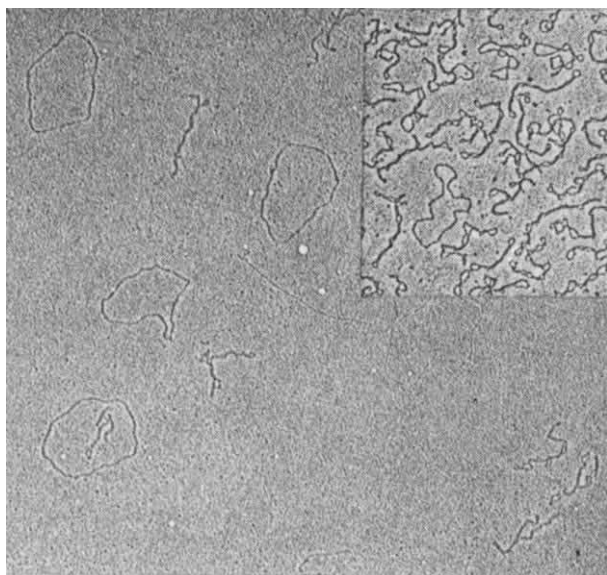


Fig. 1 Electron micrographs of T4 endonuclease-treated SV40 DNA. The figure shows unirradiated DNA (inset) consisting of 88% closed molecules and a typical irradiated sample consisting of closed and open circles. SV40 DNA was obtained by phenol extraction of purified virus isolated from infected green monkey kidney cells and was shown by electron microscopy and sedimentation analysis to consist of about 90% supercoiled molecules (form I) and 10% open circles (form II). Ultraviolet-irradiation of the DNA was performed with a 15 watt GE low pressure mercury-vapour ultraviolet germicidal lamp at a dose rate of 8 ergs/mm²/s. The dose rate was measured with a photovoltaic dose-rate meter constructed by Dr John Jagger, as well as with a Kettering model 65 radiometer (Yellow Spring Instruments). Both meters gave identical dose rates for our lamp. For most experiments DNA was irradiated at doses between 0–200 ergs/mm². The T4 excision repair endonuclease was purified as previously described⁴. The purified enzyme was stored in 0.05 M Tris-HCl buffer, pH 8.0, with 3% polyethylene glycol, at 4° C. Enzyme reactions were carried in a total volume of 37.0 µl. containing 0.8 µg SV40 DNA; 0.05 M Tris-HCl, pH 8.0; and 5 U of endonuclease. MgCl₂, when added, was at 10⁻³ M. Incubations were at 37° C for 15 min. These incubation conditions result in a complete reaction with DNA containing as much as 50 dimers per molecule⁴. Immediately following incubation the DNA was prepared for electron microscopy by a modified aqueous Kleinschmidt technique as described previously⁷. Molecules assuming an open conformation were assumed to have at least one single-strand break (form II). Molecules assuming a tightly twisted conformation were assumed to be covalently closed (form I). The stock SV40 DNA was examined simultaneously with every ultraviolet experiment and remained approximately 90% form I. (× 21,000.)

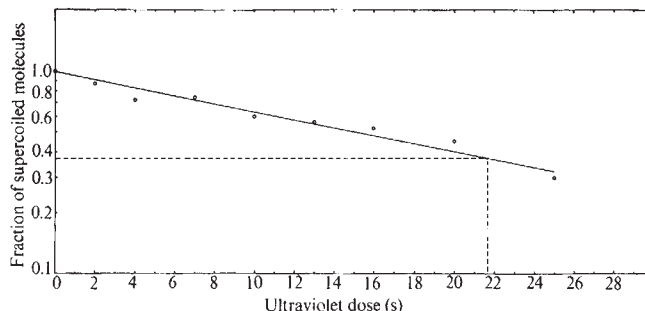


Fig. 2 Distribution of form I SV40 molecules after incubation with endonuclease as a function of ultraviolet dose. Conditions of incubation and electron microscopic scoring of molecules are described in the text. (The solid line through the data points was constructed by the least squares method.) The broken line refers to the dose of ultraviolet-irradiation at which 37% of the molecules are in the form I state.

5.06 dimers/*E. coli* genome. The dimer content/erg of the *E. coli* genome has been previously quoted in the literature as 5.0⁸, 5.2⁵ and 6.0⁹ and for the T4 genome (molecular weight of 130 × 10⁶) as 0.23⁴. As the T4 genome is 21.53 times as small as that of *E. coli* the value of 0.23 extrapolates to 5.0 for a molecular weight of 2.8 × 10⁹.

The correlation between the expected and observed number of dimers/SV40 DNA molecule suggests very strongly that every endonucleolytic incision occurred at a pyrimidine dimer site. At the very low doses of ultraviolet irradiation used, the probability of photoproducts other than dimers producing the same statistical coincidence is extremely unlikely. Finally, it is reassuring to find that a method of measuring the dimer content of DNA, which does not involve the use of radioisotopes and which does not require extensive procedural handling of the DNA, yields a result that is identical to that obtained by the older technique. The pyrimidine dimer specificity of the T4 excision-repair endonuclease may facilitate the use of this enzyme as an analytical tool for the detection of very low concentrations of dimers in DNA in a variety of photobiological studies.

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Metabolism of Polychlorinated Norbornenes by *Clostridium butyricum*

STUDIES of the metabolism of cyclodien-insecticides, such as dieldrin, endrin, endosulphan, have revealed valuable information about their fate in the ecosystem¹. With all metabolites

identified so far, however, the hexachlorinated norbornene moiety remains unchanged^{2,3}. Only from photodieldrin, a cage-like derivative of dieldrin, have dechlorinated metabolites been reported^{4,5}.

We report here the conversion of the hexachloro-norbornene moiety by a microorganism. As a result of the bicyclic structure of the hexachloro-norbornene molecule, dechlorination reactions are unlikely to occur. Any conversion products formed will probably undergo further reactions, thus preventing their accumulation and making their analytical detection in the environment difficult. Additional difficulties will be encountered by the normal metabolism of the side chains, as in the case of endosulphan⁶.

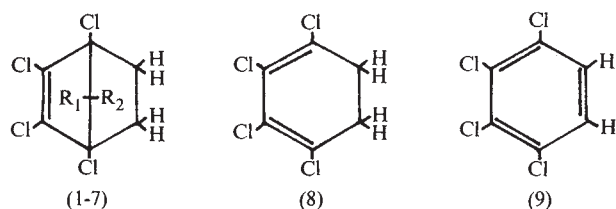


Fig. 1 Model compounds used in this study. See Table 1 for complete structures for compounds (1-7).

We started our study on the assumption that the geminal chlorine atoms at the methylene bridge of the norbornene molecule would be the most likely site for an attack by microorganisms⁷. To avoid any complications by conversions at the unchlorinated part of the norbornene derivatives we used as model compounds the unsubstituted 1,2,3,4,7,7-hexachloro-norborn-2-en (1) and its dechlorinated derivatives (2) and (3). As reference compounds for the identification of possible metabolites we synthesized the compounds (4)-(9)⁷⁻¹¹ (Fig. 1 and Table 1).

Table 1 Structures of the Model Compounds

Compound	R ₁	R ₂	Melting point (°C)
(1)	Cl	Cl	39-40*
(2)	Cl	H	59-60*
(3)	H	H	44-45*
(4)	H	OH	—†
(5)	OCH ₃	OCH ₃	28-29†
(6)	OH	OH	50-51†
(7)		C=O	100-101†
(8)	—	—	21-22‡
(9)	—	—	47-48

* See ref. 8. † Boiling point 122° C/2 torr. ‡ See ref. 7.

The polychlorinated norbornenes (1), (2) and (3) have been given in concentrations varying from 0.1-1,000 p.p.m. to cultures of the bacterium *Clostridium butyricum* growing on potatoes, covered with water in an atmosphere of nitrogen. At the end of an experiment the whole culture medium was homogenized and extracted with toluene. The resulting solution was used for electron capture gas chromatography. Culture and potatoes gave no interfering peaks in the chromatogram. For separation and identification by reference compounds three liquid phases were used: QF-1, XE-60 and OV-225 (ref. 7). Conditions for gas chromatography were the following: all duranglass column internal diameter 2 mm; length 1 m; column temperature 132° C; ⁶³Ni-electron capture detector; purge gas argon-methane⁷.

After application of the hexachloro-norbornene (1), we found a lot of starting material and the compounds (3), (4) and (8). Applying the pentachloro-norbornene (2) to the bacterium culture we could identify the compounds (3) and (4) as conversion products, whereas feeding the tetrachloro-norbornene (3) only (4) could be detected besides the starting material. Fig. 2 summarizes the results. Surprisingly the tetrachloro-cyclodien (8) could only be found when (1) was

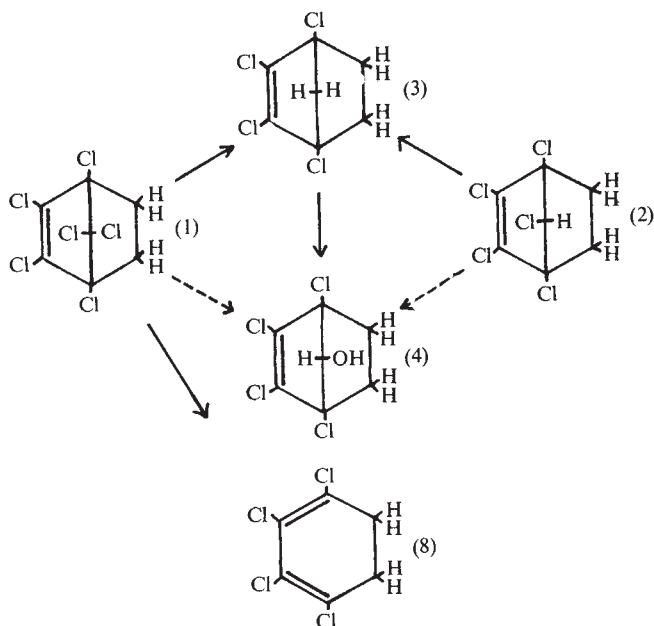


Fig. 2 Metabolism of the hexa, penta, and tetrachloro-norbornene by *Clostridium butyricum*.

given to the bacterium culture, suggesting a direct step from (1) to (8) by formally losing a dichlorocarbene moiety. So far we cannot offer a reasonable explanation for this reaction or strictly exclude that the dien (8) is an artefact from a so far unknown metabolite. Since we do not have the anti-pentachloro-norbornene isomer of (2), we cannot decide whether the formation of the alcohol (4) proceeds through this isomer or always involves the oxidation of the tetrachloro-norbornene (3). There was no evidence for the formation of the 7,7-dihydroxytetrachloro-norbornene (6) or the 7-oxo-tetrachloro-norbornene (7) respectively. The latter could be a precursor for the dien (8), as 7-oxonorbornenes can give off the bridging oxo group as carbon monoxide^{10,12}.

No dechlorinated products of the hexachloro-norbornene could be found using well-aerated yeast (*Saccharomyces*) cultures or soil microorganisms, mostly actinomycetes, on agar plates. To what extent the dechlorination reactions described here can be generalized for cyclodien-insecticides or can be found with other microorganisms is under investigation.

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Genetic Transfer of Nitrogen Fixation from *Klebsiella pneumoniae* to *Escherichia coli*

TRANSFER of nitrogen fixation genes (*nif*) within *Klebsiella pneumoniae* has been accomplished by transduction¹ and conjugation². These reports have all concerned intra-strain crosses: the transfer of *nif* to mutants of the same strain deficient in nitrogen fixation. We now report the intergeneric transfer of *nif* from *K. pneumoniae* to *Escherichia coli*, a species which does not naturally fix nitrogen.

The donor strain of *K. pneumoniae* M5a1 was prototrophic and carried a derepressed mutant I-like R factor, R144drd3 (*Km* coll)³. A derivative of M5a1 (R144drd3) was isolated following ultraviolet irradiation and subsequent selection for high frequency transfer of *his*, a marker known to be close to *nif* on the *Klebsiella* chromosome^{1,2}. This derivative, designated HF3 (R144drd3), gave rise to *his* recombinants 20 to 30 times more frequently than M5a1 (R144drd3). The recombination frequencies of a number of other auxotrophic markers were lower than *his* and diminished as the distance from the histidine operon increased. These observations suggest polarized transfer of chromosomal markers. HF3 (R144drd3), however, resembled other R factor⁴ and Col factor⁵ high frequency chromosome donors in that the autonomous sex factor still persisted. A detailed account of the behaviour of R144drd3 in *K. pneumoniae* will be published later.

In order to avoid the barrier of host specific restriction^{6,7}, which often arises in intergeneric bacterial matings, we decided to use *E. coli* strain C as a recipient, because this strain is naturally non-restricting and non-modifying⁶. Because the anaerobic procedure for direct selection of *nif*⁺ recombinants has several disadvantages, we decided to utilize the close linkage of *his* and *nif* in selecting *E. coli* *his*⁺ hybrids and screening these for inheritance of *nif*. A *his* auxotroph of *E. coli* C, strain C-603 (*his* arg *Str*) was provided by Professor G. Bertani.

Matings between *K. pneumoniae* HF3 (R144drd3) and *E. coli* C-603 were carried out at a donor : recipient ratio of 1 : 5 in unshaken nutrient broth at 37° C for 4 h and 18 h. Matings were also conducted on a solid medium using the mating technique of Matsumoto and Tazaki⁸. After mating, the mixtures were plated on arginine-supplemented minimal medium containing 250 µg streptomycin/ml. which counter-selected the donor strain. *His*⁺ colonies appeared at the low frequency of approximately 10⁻⁷ per donor cell, irrespective of mating procedure (the spontaneous reversion frequency of the *his* marker in *E. coli* C-603 was about 10⁻⁹). A low yield of marker recovery is a common feature of intergeneric matings and has been attributed to differences in nucleotide sequence between the parent chromosomes⁹⁻¹¹. *K. pneumoniae* shows only about 20% polynucleotide sequence homology with *E. coli*⁸ (personal communication from Professor S. Falkow) and this poor homology could perhaps account for the low hybrid yields observed.

Phenotypically *his*⁺ colonies were purified by restreaking twice on the selective medium and were also screened for prototrophy on minimal medium. All prototrophic isolates, which could have been streptomycin-resistant mutants of *K. pneumoniae*, were discarded. The remaining presumptive *E. coli* *his*⁺ hybrids were tested anaerobically for acetylene reduction in NH₄⁺ free medium supplemented with arginine, using the Pankhurst tube technique¹². In two experiments, ten out of twelve and two out of six *his*⁺ hybrids gave positive acetylene reduction. None of the hybrids grew well or reduced acetylene in the absence of arginine, indicating that the hybrids retained the *E. coli* arg⁻ phenotype. Nine spontaneous *his*⁺ revertants of *E. coli* C-603 did not reduce acetylene.

All the hybrids showed reactions typical of *E. coli* and could easily be distinguished from the donor strain of *K. pneumoniae*. Unlike the donor strain, all lacked lysine decar-

boxylase, were citrate and Voges-Proskauer negative and did not ferment inositol, sucrose or amygdaline (Table 1). Although the hybrids and the recipient *E. coli* strain, C-603, cross-reacted, on an Ouchterlony plate¹³, with antiserum prepared to *K. pneumoniae* M5a1 whole cells, they all gave similar patterns which differed from the donor *Klebsiella* HF3 (R144drd3) and wild-type M5a1 in lacking one major and one minor precipitation band. These properties identify the hybrids unequivocally as strains of *E. coli*.

Table 1 Cultural Characteristics of *K. pneumoniae* and *E. coli* Strains

Test	<i>K. pneumoniae</i> M5a1 (HF3-R144drd3)	<i>E. coli</i> C-603 <i>nif</i> ⁺ hybrids			<i>E. coli</i> C-603
		M8	M7	L4	
ONPG	+	+	+	+	+
Arginine dihydrolase	—	—	—	—	—
Lysine decarboxylase	+	—	—	—	—
Ornithine decarboxylase	—	—	—	—	—
Citrate	+	—	—	—	—
H ₂ S	—	—	—	—	—
Urea	—	—	—	—	—
Tryptophan	—	—	—	—	—
Indole	+	+	+	+	+
Voges-Proskauer	+	—	—	—	—
Gelatine	—	—	—	—	—
Glucose	+	+	+	+	+
Mannitol	+	+	+	+	+
Inositol	+	—	—	—	—
Sorbitol	+	+	+	+	+
Rhamnose	+	+	+	+	+
Sucrose	+	—	—	—	—
Melibiose	+	±	±	±	±
Amygdaline	+	—	—	—	—
Arabinose	+	+	+	+	+

Single colonies from 18 h nutrient agar cultures were suspended in 4 ml. sterile distilled water containing 50 µg L-histidine/ml. and 50 µg L-arginine/ml. Aliquots (0.15 ml.) of this suspension were inoculated into ampoules containing sterile test reagents (API system for identification of Enterobacteriaceae, Hughes and Hughes Ltd, Brentwood, Essex).

+, Positive. ±, doubtful positive, —, negative.

Table 2 shows the specific acetylene-reducing activity of three of the hybrids; none was as active as the donor strain but hybrid M7 approached it in activity. Table 3 shows a direct demonstration of nitrogen fixation using ¹⁵N₂, in which the total amounts of ¹⁵N₂ fixed by the hybrids were compared with that fixed by the donor strain; the fact that hybrid M8 exceeded the donor strain in the test is probably fortuitous. When grown in Pankhurst tubes containing media supplemented with 100 µg N/ml. as ammonium succinate, acetylene reduction by all hybrids was completely repressed, indicating

Table 2 Comparison of Acetylene Reduction in *K. pneumoniae* and *E. coli* C *nif*⁺ Hybrids

Organism	nmol ethylene formed/min/mg dry weight
<i>K. pneumoniae</i> M5a1 (HF3-R144drd3)	36.3
<i>E. coli</i> C-603	<0.1
<i>nif</i> ⁺ hybrid M8	12.8
<i>nif</i> ⁺ hybrid M7	20.2
<i>nif</i> ⁺ hybrid L4	7.4

Single colonies grown anaerobically on NH₄⁺ free glucose agar containing histidine and arginine (100 µg each/ml.) were inoculated into 10 ml. nutrient broth. After overnight incubation at 30° C, 1 ml. of broth culture was added to 10 ml. of NH₄⁺ free liquid medium supplemented with 100 µg histidine/ml. plus 100 µg arginine/ml. in Pankhurst tubes¹². Induction of nitrogenase was monitored at frequent intervals by acetylene reduction. When maximum activity was obtained 1 ml. of each culture was removed and the acetylene reduction rate measured under argon at 30° C in sealed 8 ml. serum bottles containing 0.5 ml. acetylene.

that the control genes as well as structural *nif* genes had been introduced into *E. coli*.

After anaerobic incubation on NH_4^+ free agar plates, containing 100 $\mu\text{g}/\text{ml}$. each of histidine and arginine, most of the hybrids formed large mucoid colonies and segregated tiny *nif*⁻ clones. Hybrids L4 and M8 also lost the ability to reduce acetylene on testing in Pankhurst tubes after one passage in nutrient broth and one on nutrient agar. Hybrid M7 appeared to be stable, however: it retained *nif* after two passages on nutrient media and did not segregate *nif*⁻ colonies during several successive platings on NH_4^+ free medium although it habitually produced slightly smaller colonies than the nitrogen-fixing colonies of the other hybrids. Thirty-seven single colonies of hybrid M7 taken from nutrient agar plates grew well anaerobically in amino-acid-supplemented NH_4^+ free medium and reduced acetylene.

Table 3 Fixation of $^{15}\text{N}_2$ by *K. pneumoniae* and *E. coli nif*⁺ Hybrids

Organism	Atom% ^{15}N excess
<i>K. pneumoniae</i> M5a1 (HF3-R144drd3)	1.883
<i>E. coli</i> C-603	-0.040
<i>nif</i> ⁺ hybrid M8	2.037
<i>nif</i> ⁺ hybrid M7	0.593
<i>nif</i> ⁺ hybrid L4	0.256

Colonies grown anaerobically on a NH_4^+ -free glucose agar supplemented with histidine and arginine (100 μg each/ml.) were inoculated into 2 ml. of similar liquid medium but containing 50 μg arginine/ml. and 50 μg histidine/ml. in 20 ml. tubes. The tubes were fitted with a pyrogallol plug and incubated for 1.5 h, after which 4 ml. gas was removed and 8 ml. 99% $^{15}\text{N}_2$ was injected. After 72 h at 30° C, the cultures were digested in a quartz Kjeldahl tube until nearly clear using 1 ml. of 2 N H_2SO_4 containing 0.05 ml. 100 vol. H_2O_2 and 0.2% CuSeO_3 . The tubes were fitted to a vacuum line and heated with excess CuO to generate N_2 . The residual CO_2 was frozen out and the remaining gas was examined for mass 29 : 28 ratio in an AEI MS10 mass spectrometer.

It is now evident that both regulatory and structural *nif* genes have been transferred to *E. coli*, possibly indicating that these loci are associated together as a *nif* operon, linked closely to *his*. The unequivocal transfer of *nif* to a new genus, seemingly stable in the case of hybrid M7, opens numerous possibilities for its transfer among other prokaryotes, particularly if the problem of host specific restriction can regularly be overcome. At present such transfer is limited by host range of sex factors and transducing phages and by differences in nucleotide sequence homology between donor and recipient chromosomes. An important step would be to obtain *nif* in the plasmid state in order to facilitate its transfer and for this purpose more must be learned of the physical state and mode of replication of *nif* DNA in *E. coli*. The recent isolation of closed circular DNA from partially diploid *Salmonella typhosa* hybrids¹⁴ may indicate that hybrid genes are commonly conserved as closed circular elements.

The establishment of *nif* genes in *E. coli* raises the questions of whether the nitrogenase proteins are identical to those in *K. pneumoniae*, whether specific electron transport proteins are coded for by *Klebsiella* DNA in hybrids and whether the recently discovered *E. coli* ferredoxin¹⁵, which is formed anaerobically, will serve. Further genetic mapping of the determinants related to *nif* on the *Klebsiella* chromosome will be necessary before such problems can be fully resolved.

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Locomotor Behaviour in Living and Fossil Pongids

LEWIS has suggested¹ that certain morphological features common to the wrist joints of living hominoids (and one fossil) were adaptations for arm swinging or brachiating behaviour and has indicated a common "brachiating" ancestry among the great apes and man. Lewis's descriptive anatomy is excellent; but we question his interpretation of hominoid locomotor evolution. The correlation between the intra-articular meniscus and brachiating abilities is not so apparent as Lewis suggests.

No matter how the locomotor patterns of gibbons and the African apes are defined, field studies have made it clear that the locomotor behavioural repertoires of the two groups are quite different²⁻⁵. The "brachiation" which Lewis describes¹ is the behaviour of the gibbon, but it does not describe the locomotor behaviour of the African apes. (In this communication "brachiation" and "brachiating" refer to gibbon type arm swinging.) Although both chimpanzees and gorillas may be considered partly arboreal in that they often feed and nest in the trees (particularly chimpanzees), their normal mode of progression is not to be confused with the "ricochetal arm swinging" shown of the gibbon. Both chimpanzees and gorillas are "fundamentally semi-erect quadrupeds and assume knuckle walking postures both on the ground and on stout horizontal branches, even high in the trees"⁶. Tuttle, one of the few workers to appreciate the unique locomotor pattern, knuckle walking, of the African great apes, has presented detailed evidence that the wrist joints and hands of the African pongids are uniquely adapted for this type of locomotion⁶⁻⁸. Similarly, Lewis's comparative work, showing that the wrist of the gibbon, the only undoubted brachiator, differs from that of the African great apes^{1,9}, would suggest that the wrists of *Pan* and *Gorilla* are not adapted for brachiation. It should also be noted that those monkeys which have been dubbed "semi-brachiators" such as *Ateles* or *Presbytis* (the former definitely shows suspensory locomotion) apparently show no tendency to develop the type of wrist joint which Lewis associates¹ with brachiation, in other words, that found in the African apes.

It is difficult to agree with Lewis when he states that "the wrist joint (after the disengagement of the styloid process of the ulna from the carpal bones) is now less fitted for supportive functions . . ."⁹. Such a statement is not supported by the fact that the largest living primate, *Gorilla*, transmits much of its considerable body weight through a wrist which lacks an ulna-carpal articulation. It seems more compatible with the field studies to regard both the presence of the wrist meniscus and the reduction of the styloid process as morphological

features allowing maximum flexibility at the wrist joint without necessarily reducing its ability to withstand compressive forces during locomotion. Such an interpretation also accords well with occurrence of interarticular fibro-cartilaginous menisci elsewhere in the body (for example, temporomandibular, sternoclavicular, knee joints)¹⁰.

Why, then, should the gibbon, brachiator *par excellence*, have the most cercopithecoid-like wrist joint among the hominoids? Perhaps the gibbon wrist is adapted for stability in order to sustain the constant tensile shocks involved in rapid arm swinging. As Lewis points out⁹, the bony lunula serves to stabilize the wedge-shaped meniscus, and this lunula is most prominently developed in gibbons.

Lewis's evidence^{1,9} as to the "advanced" (that is, non-gibbon-like) hominoid wrist of *Dryopithecus (Proconsul) africanus* (from the early Miocene of Rusinga Island, Kenya) should remove all doubt about the hominoid status of this fossil ape, both dentally and postcranially. That the Miocene apes were not cercopithecoid-like postcranially is shown not only by the *D. (Proconsul) africanus* material, but also by the humerus of *D. fontani*¹¹ from St Gaudens in France, and the ulna, probably of the latter species (originally referred to as *Austriacopithecus weinfurteri*) from the Miocene of Austria¹². As Lewis notes¹, the wrist of *D. (Proconsul) africanus* differs more from cercopithecoid wrist morphology than does the modern gibbon wrist. This would imply that by early Miocene times the locomotor patterns characteristic of gibbons and the African apes were already distinct (unless it were postulated that the "advanced" wrist of Miocene *D. (Proconsul) africanus* subsequently reverted to a more cercopithecoid-like wrist of the sort seen in the modern gibbon). It would also argue against the idea that *D. (Proconsul) africanus* was a "pro-brachiator" ancestral to later brachiating forms.

Other features in the forearm and wrist further suggest that *D. (Proconsul) africanus* was more quadrupedal than Lewis implies, and probably more terrestrial than suggested by Napier and Davis¹³. In the humerus, the prominent deltopectoral crest, the posteriorly directed medial epicondyle, the anterior convexity of the shaft and the medial slope of the inferior articular surface (A. Walker, personal communication) are all features which are found among terrestrially quadrupedal primates. That the quadrupedalism of *D. (Proconsul) africanus* might not have been palmigrade, as in cercopithecoids, is suggested by the absence of deep grooves on the posterior surface of the distal part of the radius for the extensor muscles of the carpus, as well as by the overall similarities in the wrist¹ and elbow joints of *D. (Proconsul) africanus* and the African pongids. There is thus the distinct possibility that by Miocene times (18 million yr BP) incipient knuckle walking was already evolving in at least one lineage of African apes.

If this view is correct, then man had a brachiating ancestry only if brachiation evolved before early Miocene times, that is, before the locomotor patterns characteristic of gibbons and the African pongids differentiated. This, of course, assumes, as all anatomical and biochemical evidence would suggest, that man is more closely related to the African apes than to the gibbon.

If Lewis's suggestion¹ is followed that the advanced hominoid wrist (as seen in *Pan* and *Gorilla*) is a brachiating adaptation, it would seem that the only primates which consistently brachiate are ill adapted for such activity and the knuckle walking *Gorilla* possesses brachiating adaptations which it does not use. Rather, the evidence from the fossil postcranial material combined with modern behavioural analogies suggests that the morphology of the "advanced" hominoid wrist is correlated more with knuckle walking and the necessity for a flexible hand (as in all hominoids) than with gibbon type brachiation. This flexibility, which is attributable to the reduction of the ulnar styloid process, allows a greater range of hand adduction toward the ulna side of the wrist (such adduction is necessary in any knuckle walking posture in which the hands are not placed perpendicular to the long axis of the body).

That some knuckle walking was probably a part of the total locomotor repertoire of *D. (Proconsul) africanus*, the subsequent African pongids and possibly early hominids is suggested by the numerous similarities to *Pan* seen in the Miocene dryopithecines.

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Epidemiological Model for Passive Transfer of IPNV by Homeotherms

INFECTIOUS pancreatic necrosis virus (IPNV) is a highly infectious virus of salmonid fishes, which has been isolated from virtually every trout-producing area of the world. Infections have been transmitted through water¹, by injection² and by protozoan parasites³. Wolf *et al.*⁴ established the important epidemiological concept of vertical transmission when they demonstrated that eggs from carrier brood stock developed epizootics of IPN. We have now investigated whether free living warm blooded animals which have eaten IPNV-infected animals can possibly transfer infectious virus or become actively infected with the agent.

We used the rainbow trout gonad cell line (RIG-2) established by Wolf and Quimby⁵ to detect IPNV infectivity and to produce virus inoculum. The baby hamster kidney cell line (BHK-21), established by MacPherson and Stoker⁶, was inoculated with 10⁶ TCID₅₀ of IPNV and subcultured three times (blind passage), when confluent cell monolayers were obtained. The IPNV strain was obtained from an Ontario government provincial hatchery, and identified by serum neutralization and electron microscopy.

We inoculated about 10⁸ TCID₅₀ IPNV into chicken, great horned owl (*Bubo virginianus*), great blue heron (*Ardea herodias*), common eider (*Somateria mollissima*) and American merganser (*Nergus merganser americanus*) using a syringe and an 18 gauge needle fitted with a 4 inch piece of rubber tubing. The mouth was forced open, the rubber tubing inserted down the oesophagus, and the virus suspension introduced directly into the digestive tract. Mink (*Mustella* sp.) were inoculated by feeding them on fish which have been injected with IPNV.

Four hours after inoculation, clean kraft paper was placed under the caged animals and faecal samples were collected at 4 h intervals for 24 h. Samples were assayed for virus by the method of Wolf *et al.*⁷. The great blue heron and mink were killed 7 days after inoculation and virus was assayed in the spleen by the procedure of Hoffman *et al.*⁸. IPNV infectivity was detected in the faeces of chicken, great horned owl, and mink, but not in those of the American merganser, common eider or great blue heron. No IPNV was detected in the spleen samples. There was no evidence of cytopathic effect in the BHK-21 cell line inoculated with IPNV.

The isolation of IPNV from the faeces of inoculated chicken, great horned owl and mink suggests that a wide variety of warm blooded animals could be "mechanical vectors" of the virus. During the spring and autumn, many birds migrate from the south to the north and back again, and could cause IPNV epizootics far from the natural foci of infection. We found no evidence that mink and great blue heron were infected with IPNV. Similarly, attempts to adapt IPNV infectivity to warm blooded cell lines, as reported here and by Wolf *et al.*², failed. Angiolielli and Rio⁹, however, reported evidence of IPNV infectivity in inoculated suckling mice and suggested that mice may become infected with IPNV and might act as reservoirs of the disease.

The passive "mechanical vector" mechanism has also been suggested as a mode of spread of whirling disease of salmonid fishes caused by the protozoan (*Myxosoma cerebralis*)¹⁰.

As our water resources are more intensively utilized for aquaculture, it is becoming increasingly important that we become aware of the problems that IPNV (and other fish pathogens) may cause in these waters. If a specific pathogen-free population of fish is acquired for aquaculture, their continued protection could be jeopardized. The possibility that free living wildlife (poikilotherms and homeotherms) may transfer or be infected with agents pathogenic for hatchery fish (and vice versa) cannot be overlooked.

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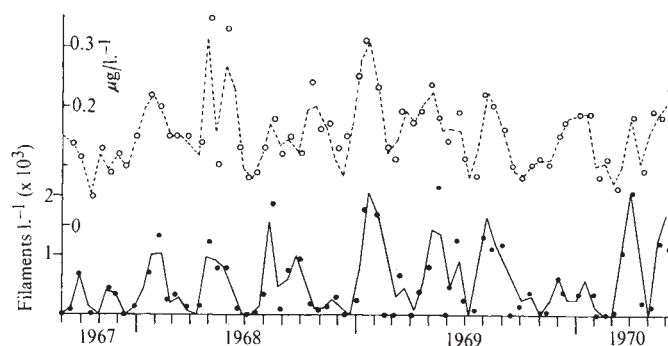


Fig. 1 Chlorophyll concentration (○---○) and numbers of *Trichodesmium* (●—●) at 5 m. Circles show the observed values, lines the interpolated 15 day interval values.

obtained from occasional cruises and there are relatively few extended series of year-round observations from specific localities. We have observed a hitherto unrecognized type of cycle; oscillatory variations of relatively short periodicity that are apparently independent of the annual solar cycle.

Our material was collected during a more comprehensive investigation of biological production in the tropical Western Atlantic. Sixty-five collections were made at approximately fortnightly intervals from August 29, 1967, to June 4, 1970, at an oceanographic station situated at latitude 13° 15' N, longitude 59° 43' W, about 9 km west of Speightstown, Barbados, in a depth of 460 m. Although relatively close to the island it has been shown that the influence of land on the biological production system is negligible and conditions are considered to be typical of this part of the Atlantic Ocean⁵. Chlorophyll concentration was measured at 5, 25, 50, 75 and 100 m in 2 litre samples by the modified method of Richards and Thompson as given by Strickland and Parsons⁶. The numbers of phytoplankton were estimated at 5, 50 and 100 m in 0.5 l. samples of water fixed with 1% neutralized formalin. After being allowed to settle for several days in tall glass cylinders the samples were reduced to 25 ml. by very slow siphoning and were examined with a Zeiss Utermohl inverted microscope. The number of filaments of *Trichodesmium thiebautii* and other common phytoplankton, consisting chiefly of several species of widely distributed diatoms, were counted.

Inspection of the raw data showed that chlorophyll concentration and the number of filaments of *Trichodesmium* at 5 m fluctuated regularly with a periodicity of between 3 and 4 months. Other types of phytoplankton were usually less numerous and did not vary in this way. About 0.1 µg l.⁻¹ of chlorophyll could be ascribed to other species while amounts in excess of this seemed to be associated with the variations of the *Trichodesmium* population. This association was studied by harmonic analysis and by auto and cross-correlations. Estimated values for chlorophyll and *Trichodesmium* at 15 day intervals were derived by interpolation between the observed values using a fourth order polynomial and are shown, together with the observed values, in Fig. 1. To reduce the effect of fluctuations other than the dominant one, the interpolated data were smoothed with a digital filter

$$S_F(t) = 0.25 - S_{(t+2n)} + 2S_{(t)} - S_{(t-2n)} \quad (1)$$

having a gain function

$$G(f) = \sin^2(2\pi n f) \quad (2)$$

where S_F represents the filtered values, S the interpolated values, t the number of observations, f the frequency of oscillation and G the gain of the filter.

The effect of this transformation is shown in Fig. 2 which reveals clearly that chlorophyll and *Trichodesmium* oscillate in phase with a periodicity of about 105 to 120 days. The filter shortens the series at each end by $2n$ samples reducing the number of pairs of interpolated values from 68 to 60. Fifty-six pairs of values, forming a continuous series from the 60th

Oscillatory Variation of a Phytoplankton Population in a Tropical Ocean

PRODUCTION in permanently stratified tropical seas removed from immediate land influence is believed to be either low and relatively uniform throughout the year^{1,2} or higher in the winter than in the summer months^{3,4}. Most models of the production cycle in tropical oceans are based on informaton

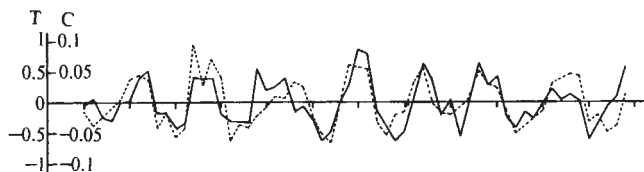


Fig. 2 In phase variation of chlorophyll and *Trichodesmium* after smoothing by digital filter. Horizontal axis is marked at 75 day intervals: T, thousands of filaments/l. of *Trichodesmium*; C, µg/l. of chlorophyll.

to the 885th day, were used for the harmonic analysis, equal numbers of interpolated and filtered values being subjected to the same procedure. The amplitudes obtained from the filtered series were divided by the gain of the filter. This analysis, presented in Table 1, also shows that the dominant oscillation has a period of 120 days and that chlorophyll concentration and numbers of *Trichodesmium* are very well in phase. The same conclusion was reached by calculating the auto and cross-correlations between *Trichodesmium* (1) and chlorophyll (2). These functions are shown graphically in Fig. 3. Filtering the interpolated data greatly increased the auto-correlation but the cross-correlation only moderately. The unfiltered data, however, already showed a high degree of cross-correlation so that the smoothing procedure would not be expected to give much increase of this correlation.

A similar analysis was applied to the combined data from all depths to 100 m. Oscillations of both components were less apparent and were out of phase, and both auto and cross-correlation functions were considerably less, such correlation as was present being due mainly to the excellent agreement at 5 m. The reason for this is clear when the depth distribution of chlorophyll and *Trichodesmium* is examined (Fig. 4). *Trichodesmium* is characteristically most abundant near the sea surface, more than half the total number of filaments being at 5 m, while chlorophyll concentration increases with depth, being greatest at 75 m about the level of the shallow thermocline in this part of the ocean. We may conclude therefore that with increasing depth a larger fraction of the chlorophyll is associated with organisms other than *Trichodesmium*.

These findings raise the question of how the population cycle of *Trichodesmium* is regulated. There is little direct evidence. The oscillations cannot at present be linked with any effect of the annual solar cycle operating through variations in the hydrographic conditions. Annual variations of day length, surface water temperature and salinity are small in

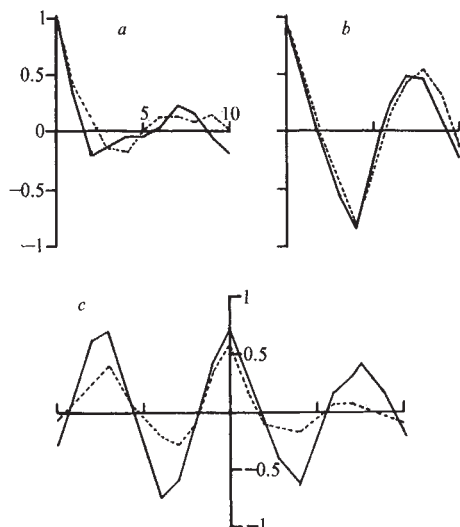


Fig. 3 a, Auto-correlation of interpolates; b, auto-correlation of filtered series; c, cross-correlations of interpolated series (—) and filtered series (---). Horizontal axes show ten 15 day intervals.

Table 1 Harmonic Analysis of Interpolated and Filtered Values

Harmonic	Period (days)	Interpolated series			
		Chlorophyll		<i>Trichodesmium</i>	
		Amplitude	Phase	Amplitude	Phase
4	210	0.007	72	67	-165
5	168	0.007	36	178	65
6	140	0.022	11	113	-54
7	120	0.039	96	318	104
8	105	0.017	-20	147	-28
9	93	0.018	-94	295	-66
10	84	0.020	84	123	117

Harmonic	Period (days)	Filtered series			
		Chlorophyll		<i>Trichodesmium</i>	
		Amplitude	Phase	Amplitude	Phase
4	210	0.010	92	75	-144
5	168	0.006	65	168	59
6	140	0.019	13	129	-52
7	120	0.038	99	311	101
8	105	0.016	-26	162	-24
9	93	0.019	-96	291	-61
10	84	0.020	85	144	106

this part of the ocean, the water column is permanently stratified and the concentrations of nutrients in the upper layers are low at all times⁷. Our observations suggest that the growth and regeneration cycles of *Trichodesmium* near the surface may depend on events in deeper water. Characteristically *Trichodesmium* blooms at 5 m disintegrated as a mass of decaying filaments with numerous bacteria and was followed by a period, usually 2 weeks to a month, when few or no filaments were found. On several occasions considerable numbers of healthy filaments were found at 50 and 100 m 2 weeks before they reappeared near the surface. Conversely when *Trichodesmium* was abundant at 5 m it was often absent or found in small numbers only at the deeper levels.

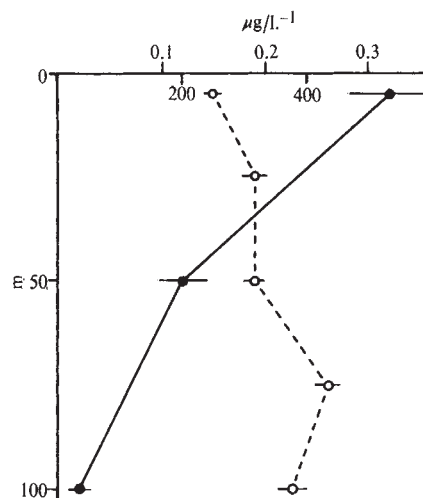


Fig. 4 Average and standard error of chlorophyll concentration (○ --- ○), and numbers of *Trichodesmium* (● — ●) 0-100 m.

We may therefore put forward a tentative hypothesis for a *Trichodesmium* cycle based on the presence of a relatively small "seed" population near the base of the euphotic zone from which the blooms at the surface are regenerated at intervals of about 120 days. If true this would suggest that in deeper water the plant has access to essential nutrients in quantities sufficient to sustain its growth in the impoverished surface water for a limited period and that the population collapses when this reserve is exhausted. What these substances may be can only be a matter for speculation at present, but it is presumably something other than nitrogen since there is considerable evidence that *Trichodesmium* can fix atmo-

spheric nitrogen⁸. This capacity would be expected to favour its growth relative to other types of phytoplankton and could explain why it is in many areas the dominant form in the surface water of tropical seas.

Finally, we may consider the oscillations of *Trichodesmium* as an example of a free-running cycle, although this may simply be a statement of our ignorance of the controlling mechanisms involved. Cycles that are independent of the solar year and seem in some respects analogous to ours have been described in the reproduction of several species of tropical sea birds, which breed at less than annual intervals apparently determined solely by biological factors such as the minimum length of time required to raise a brood, moult and complete gonad regeneration⁹.

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Passage of Bracken Fern Toxicity into Milk

BRACKEN (*Pteridium aquilinum*) and extracts of the plant have pluripotent carcinogenic and mutagenic properties, and if administered in sufficient concentration are acutely toxic to species as varied as mice and bovines¹⁻⁹. The causative factor(s) of these different biological activities seem to be identical; they are produced by the same purified fraction although obtained by different fractionation procedures^{5,10-12}. This is not surprising because the acute lethal syndrome is radiomimetic in nature, being characterized by bone marrow aplasia, haemorrhage and severe gastro-intestinal damage,

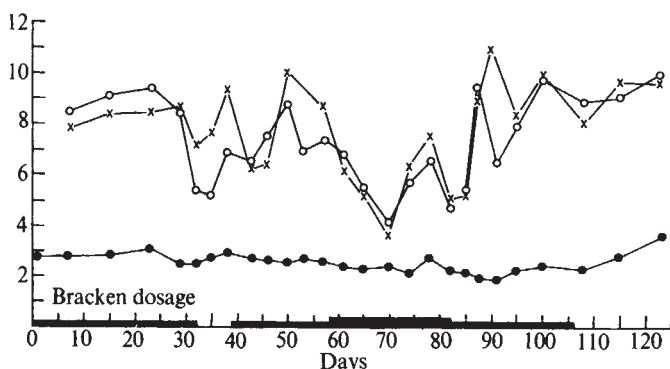


Fig. 1 Haematological response in a calf consuming milk from bracken-fed cows. $\times$, Platelets $\times 10^5 \text{ mm}^{-3}$; $\circ$, total leucocytes $\times 10^3 \text{ mm}^{-3}$; $\bullet$, micro-haematocrit erythrocytes volume %.

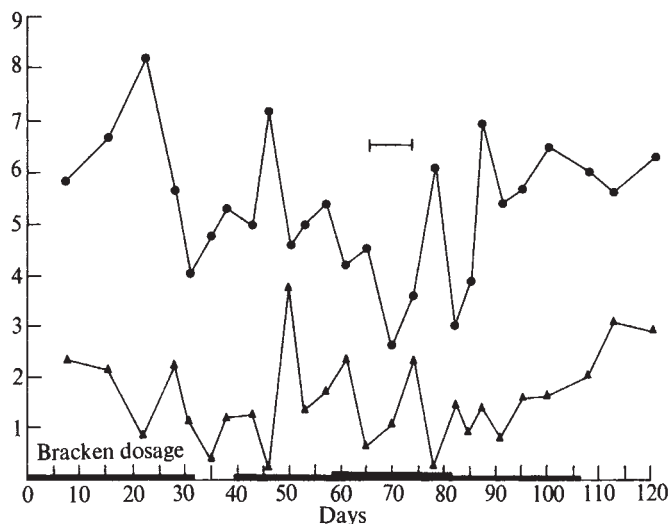


Fig. 2 Differential leucocyte count of a calf consuming milk from bracken-fed cows. $\bullet$, Lymphocytes $\times 10^3 \text{ mm}^{-3}$; $\blacktriangle$, neutrophils $\times 10^3 \text{ mm}^{-3}$ (average of 1,000 cells per count); —, circulating pyknotic normocytes.

together with other systemic changes common to both syndromes¹³⁻¹⁵.

A final stage of thin-layer chromatography has led to the isolation of a biologically active principle for which elemental analysis and mass spectrometry give a molecular formula $\text{C}_7\text{H}_{10}\text{O}_5$; it is highly water soluble and many other chemical properties have been described^{5,11,12}. This information would suggest that passage into the milk of cows ingesting bracken is possible, and the presence of a carcinogen in the urine of such animals has already been reported^{16,17}. The contamination of milk in this manner by a naturally occurring carcinogen could well constitute a possible hazard for human health in the many areas throughout the world of upland and marginal farming where cattle are known to consume bracken. Furthermore, we have demonstrated an age dependence in bracken-induced tumours in rats where it is the young animal which is at risk².

Although direct chemical tests for the presence of the toxin in bovine milk have given positive results this does not necessarily mean that it still has biological activity, since mass spectrometry results can be the same for inactive as well as active samples¹¹. The toxin in solution, especially when purified, is relatively unstable, and also the possibility of production of different metabolites in the cow must be remembered. We are therefore testing the milk from cows receiving a bracken supplement for carcinogenic activity by feeding it, both whole and processed, to different strains of rats and mice.

While doing this and waiting for the long term results, we also fed the milk to a young calf in order to investigate whether or not sufficient toxin was present in the milk to produce impairment of bone marrow activity with consequent depression of the calf peripheral blood cell count, as is found in the acute cattle syndrome.

A Friesian and an Ayrshire cow were used alternately, and in the first phase of the experiment they received a daily supplement to their normal diet of 1.4 kg of dried, milled bracken which was pelleted into reconstituted nuts and fed twice daily. Later, when we found a response in the calf, the amount of bracken fed to the cows was increased to an average of 2 kg for about 25 days in an attempt to accentuate the effects in the calf. The Guernsey bull calf was 5 weeks at the beginning of the experiment and was fed by bucket at the rate of about 1 gallon of milk a day, increasing to 1.5 gallons as it grew older. It was bled from an ear vein at intervals of roughly 4 days and the total and differential leucocytes and platelets recorded. Micro-haematocrit readings were taken using the Hawksley machine (Figs. 1 and 2).

There was a marked decrease in leucocytes at the end of about 4 weeks, followed slightly later by a decrease in platelets. There was then a recovery phase before another sharp descent to abnormally low levels at 70 days. This is a typical response given by a calf which is consuming bracken at a low rate directly. The recovery in this case took from day 70 to about day 100 and is marked by the see-saw efforts of the bone marrow.

The damage sustained by the bone marrow is shown most clearly by the neutrophil count which at two points was almost reduced to zero, and by the appearance in the circulation of pyknotic normocytes during the period of maximum depression. Normal bovine blood does not even contain reticulocytes. The erythrocyte volume fell to below 20% round about day 90 and then subsequently recovered.

From day 34 to 56 there were intermittent small amounts of blood and mucus in the faeces, which is the usual indication in experimental calves of early intestinal damage. Further support for the passage of the toxin into milk comes from an experiment with neonatal Swiss White non-inbred mice in which the mothers were fed throughout pregnancy and lactation (6 weeks) with normal diets supplemented with different bracken extracts which were incorporated into the 41B diet at a level equivalent to 1 kg of dried bracken per 3 kg diet. The offspring were prevented from eating this so that the toxin could only be transmitted to the young through maternal passage through the placenta and/or milk. They were kept for 12–18 months, which we have found to be the optimum period for mice in this system¹⁴, and scored for the presence of peripheral pulmonary adenomas (Table 1).

Table 1 Maternal Passage of the Bracken Carcinogen in Mice through the Placenta and/or Milk

Extracts given daily with food to mother mice during pregnancy and lactation	No. of animals	Offspring No. with pulmonary adenomas	%
Control	27	1	4
Alkaline ether extract	21	1	5
Alkaline ether residue	34	7	20
Methyl acetate extract	12	8	75
Methyl acetate residue	11	6	55

The extracts were prepared by methods similar to those already described^{10–12}, and the results are well in accord with the degree of toxicity of the fractions as shown by other direct tests. Little activity goes into ether and it is markedly reduced by alkaline conditions, whereas methyl acetate is a relatively good solvent, and there has obviously been genuine maternal passage of the carcinogen.

It may be objected that the numbers of animals are not large enough, so it is pertinent to mention that preliminary results from later experiments with more mice are confirming the maternal passage. In this instance we also narrowed the possibilities in two groups by transfer of litters so that the offspring could have only had the toxin either *in utero* or by the milk (3 weeks), but not both. Subject to confirmation, it seems that the milk is the principal pathway of transmission.

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Ecdysones and Locust Extracts in Plant Bioassays

It has been shown that endogenous gibberellin-like substances are translocated in the phloem of plants¹ by using the aphid *Tuberolachnus salignus* (Gmelin) to obtain sieve tube sap samples. It has also been suggested² that steroid moulting hormones of insects have effects similar to those of gibberellins in plant bioassays, and both insects and plants have been shown to contain similar steroid hormones that are active in bringing about moulting in insect assays³. In view of these findings, some of these hormones were obtained in crystalline form and tested to determine whether they are promotive in plant assays for gibberellins. The earlier work² was also repeated using extracts of whole locusts (*Schistocerca gregaria*) and their prothoracic glands.

Table 1 Phytoecdysones and Ecdysones Tested for Gibberellin-like Activity and their Source

Phytoecdysone	Plant source
Ponasterone A	<i>Podocarpus nakaii</i>
Ecdysterone*	<i>Achyranthes fauriei</i>
Inokosterone	<i>Achyranthes fauriei</i>
Cyasterone	<i>Cyathula capitata</i>
Polypodine A*	<i>Polypodium vulgare</i>
Polypodine B	<i>Polypodium vulgare</i>
Ecdysone	Animal source
α-Ecdysone	<i>Manduca sexta</i> pupae
Synthetic ecdysone analogue†	

* Identical chemically.

† 2β,3β,14α-trihydroxy-5β-cholest-7-ene-6-one.

Each of the characterized compounds (Table 1) was dissolved in absolute methanol and tested for the concentration range 100 μg ml.⁻¹ to 0.01 μg ml.⁻¹ in the following plant bioassays for gibberellins: lettuce hypocotyl⁴, excised dwarf pea epicotyl⁵, intact dwarf pea internode⁶, oat mesocotyl⁷, oat first leaf⁸ and dock leaf senescence⁹ assays. No activity was detected after application of the known compounds over this concentration range ($P < 1\%$).

Methanolic extracts of two whole locust samples were examined, one of mid fifth instar locusts and another of late fifth instar locusts. Ethyl acetate and butanol fractions of the extracts were tested for biological activity in the above assays.

The oat mesocotyl assay, which responds to both indoles and gibberellins, was the only test in which activity was recorded. Here growth promotion was considerable, being 350% that of the controls when the ethyl acetate fractions were bioassayed at a concentration equal to one locust equivalent

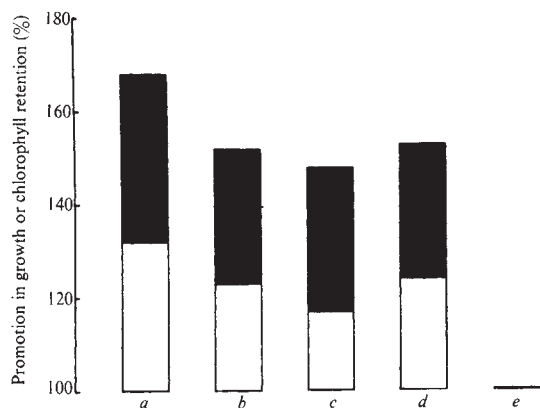


Fig. 1 Bioassays of methanol extracts of twenty prothoracic glands. *a*, Dock leaf senescence assay. *b*, Oat mesocotyl assay. *c*, Oat first leaf assay. *d*, Dwarf pea epicotyl assay. *e*, Lettuce hypocotyl assay. Chlorophyll retention (*a*) or growth (*b-e*) significantly different from the controls is indicated by the darkened area ($P < 1\%$).

per replicate. It was shown by bioassay and reaction with chromogenic sprays that the active fraction co-chromatographed with indolyl-3-acetic acid (IAA) in five solvents on thin-layer chromatography (TLC). Further evidence for the active fraction being IAA was obtained by using gas-liquid chromatography (GLC) techniques. The active fraction when eluted from the TLC plates and methylated with ethereal diazomethane had the same retention time as methyl-IAA on 2% SE 30 and 2% 'Versamid 900' columns.

The prothoracic glands of insects produce hormones which play an important part in moulting, and previous work has shown extracts of the glands to be active in gibberellin assays². In this study methanol extracts of freeze dried prothoracic glands were active in four of the bioassays (Fig. 1) confirming the presence of a compound with gibberellin-like activity. Thin-layer chromatography of the gland extract on silica gel GF₂₅₄ using chloroform : ethanol (95%) (80 : 20 v/v) as the solvent system was followed by bioassay using oat mesocotyls or *Rumex* leaf disks. A portion of the chromatographed extract was also assayed for moulting activity using isolated

locust abdomens. Using ten abdomens for each test 71.4% and 66.7% showed evidence of moulting when injected with eluates of R_F 0.10–0.18 and 0.18–0.30 respectively: the eluate of R_F 0.0–0.1 gave only 14.3% compared with 20% for the controls. The gibberellin-like activity, however, appeared in R_F 0.0–0.1 and was therefore not associated with the moulting activity present in the higher R_F , in which bands of ultraviolet absorption, a characteristic of ecdysones, were visible (Fig. 2).

Small amounts of ecdysterone have been detected in locust whole nymph extracts¹⁰. Clearly neither this nor other moulting inducing compounds present are responsible for the gibberellin-like activity shown by gland extracts in plant assays. Our results resolve the apparent paradox of plants that contain high levels of phytoecdysone without exhibiting the growth characteristics associated with high levels of gibberellin.

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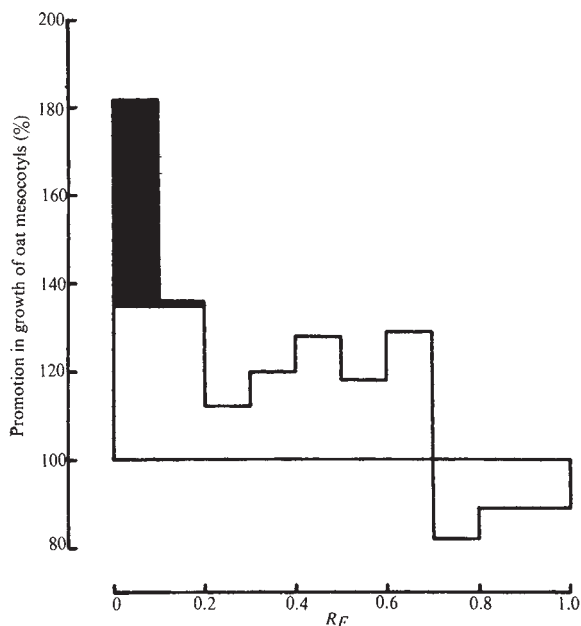


Fig. 2 Oat mesocotyl assay of a methanolic extract of twenty prothoracic glands chromatographed on silica gel GF 254 in chloroform : 95% ethanol (80 : 20 v/v). Growth significantly different from the controls is indicated by the darkened area ($P < 1\%$).

Alpha Rhythm and Eye Movements in Eidetic Imagery

SOME children and rare adults possess a remarkable ability to recall visual images in great detail¹. These eidetic images, unlike visual after-images, remain still while the eyes move and can be recalled long after after-images fade². Recently, attempts have been made to confirm their authenticity in studies wherein a subject is asked to recall an eidetic image of a specified picture, superimpose it onto a new second picture, and report detail produced by the superposition of the two images². Stromeyer and Psotka³ have utilized a similar superimposition principle using Julesz⁴ random dot stereograms.

It is not known whether eidetikers possess an alpha rhythm or whether the recall of eidetic image would block the alpha. Furthermore, little is known about eye movements during eidetic imagery⁵. We therefore investigated these questions with Stromeyer's and Psotka's eidetic subject, a 23 yr old, emmetropic, ambidextrous woman. Methods and electrode placements for recording electroencephalogram (EEG) and electrooculogram (EOG) were conventional⁶⁻⁸. Recordings were carried out on November 24, 1969.

When sitting relaxed with her eyes closed, the subject had a normal alpha rhythm which blocked when she opened her eyes (Fig. 1A). When the subject read at either 34 cm or from

slides projected onto a screen at 6 m away, her alpha rhythm blocked. As she built up an eidetic image of any detailed visual scene by scanning over the material a number of times, her alpha also blocked (Fig. 2A). When the subject recalled an eidetic image from material acquired years ago and then read it silently with her eyes closed, a large amount of alpha rhythm appeared and saccadic movements were recorded (Fig. 1C). These saccadic sequences lacked the regularity associated with the reading of new material (Fig. 1B). While the subject built up an eidetic image of new material projected onto a screen at 6 m or from the *New Yorker* at 34 cm (Fig. 2A), no alpha rhythm was present. When an eidetic image had been established, she closed her eyes and read it aloud rapidly and accurately, and the alpha amplitude (Figs. 1D and 2B) approached that of the eyes' closed resting state (Fig. 1A).

A print of Chagall's "My Village" was then placed at 6 m, and the subject proceeded to build up an eidetic image. No alpha was present during the build-up period, which lasted several minutes (see also Fig. 4C). When the subject described the image with eyes closed, the alpha rhythm returned (Fig. 2C and D). When asked to describe more details of the print, the subject accurately did so, during which period frequent brief intervals of convergence were noted (Fig. 2D). Alpha rhythm was quite prominent during this period of recall.

The subject described the painting with a speed that could scarcely have been exceeded had she been looking directly at it. She supplied the following description in 68 s: "O K, Ah. Horse and green man facing each other. Horse or cow. The man has a yellow hat with a red band. The horse is mostly pink and white except he's blue in the neck just above the necklace he's wearing. And under his eye is someone milking a cow. Up above this is another sort of sphere-like thing. Heading back is a green man carrying a hoe, I think, and a girl standing

on her head. Behind them are houses. The one on the left is yellow. It's upright. Then there's a blue one. Then there's a red one upside down, a blue one upside down, a blue one right side up, and then a yellow one right side up. There's a moon over that. The centre of the picture has a red sphere in it. Down in the bottom centre is a tree of some sort in a triangular shape with some little green blobs and a bunch of brown blobs in the branches. And the bottom left hand is mostly reds and pinks. Bottom right there's some yellow and blue. The sky at the top is black. Anything else?"

The subject could recall an eidetic image with her eyes open without difficulty, but the alpha rhythm was of reduced amplitude (Fig. 3A, B). The subject had eidetically stored a passage from Goethe's *Faust* learned in German a number of years ago. When she eidetically read this page with her eyes closed (Fig. 1C) a large amount of alpha rhythm was present. But when she placed the eidetic page on a screen at 6 m and read it with eyes open (Fig. 3A) or placed it on a blank page at 25 cm (Fig. 3B) the alpha rhythm was generally less than one-third the amplitude of the normal resting alpha rhythm or that during eidetic recall with eyes closed. Note, however, that the amplitude and incidence of this reduced alpha rhythm were much larger than the alpha rhythm recorded on opening the eyes (Fig. 1A) or during the build-up of an eidetic image (Fig. 2A). During recall of an eidetic image with her eyes open the subject had an unobstructed view of a white screen subtending $12^\circ \times 12^\circ$ at 6 m. She did not focus usually on any objects in the environment during eidetic recall and could suppress eidetic images by focusing on real objects. When the subject described features of an eidetic image, her eyes made saccadic movements in the direction of the described objects. She could bring an image closer with convergence or let it recede with divergence (Fig. 4A). On relaxation

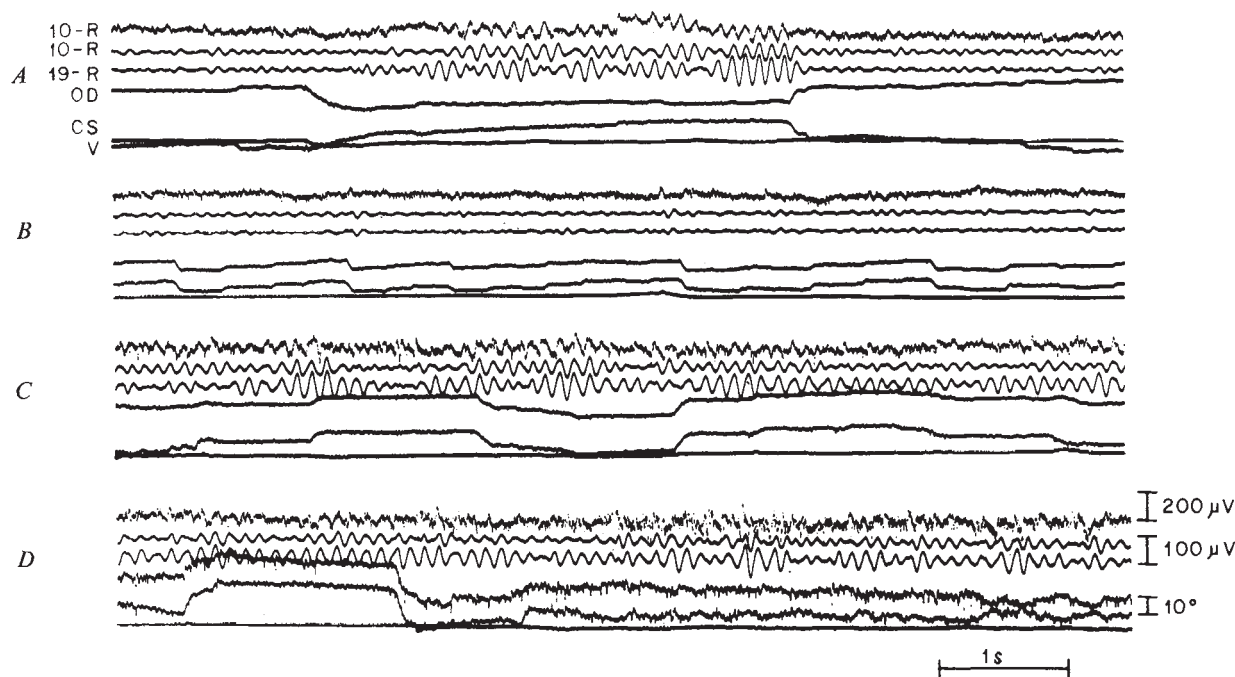


Fig. 1 A, Normal alpha rhythm appears shortly after eye closure and a normal block of the alpha rhythm occurs when eyes open. In this and all subsequent records negative polarity of the EEG traces is indicated by an upgoing deflexion. Lead 10 records from over the lateral aspect of the right occipital lobe and lead 19 records from a midline posterior occipital placement. Normal alpha could also be recorded from lead 4 over the frontal cortex and lead 8 roughly over the parieto-occipital junction. A midline back of the neck lead was reference. Top "unfiltered" EEG trace recorded at lower and upper filter settings of 0.3 and 120 Hz. The second and third EEG traces are recorded with upper and lower filter settings of 8 and 12 Hz. Because much of the study involved movement and speech by the subject, it was sometimes necessary to filter out muscle artefacts. Below and above the cutoff frequencies, signal amplitude fell off at 36 dB/octave. The fourth trace (OD) indicates the EOG of right eye with an upward deflexion indicating a movement to the right. The fifth trace (OS) indicates EOG of left eye, and in all records except A an upward deflexion indicates a movement to the right. The sixth trace is a vertical EOG trace from right eye recorded at low gain. B, EEG and EOG as subject read from page 95 of R. L. Gregory's *Eye and Brain*, McGraw-Hill, 1966, held at 34 cm from eyes. Alpha rhythm is blocked. C, Subject silently reading in German from an eidetically recalled page of Goethe's *Faust*, eidetically stored a number of years ago. Relatively large amounts of alpha rhythm are present in the EEG. Eyes are closed. D, Subject has eidetically recalled an eidetic page of newly presented material and is reading it aloud. Muscle twitch potentials from eye and face muscles may be seen on the "unfiltered" EOG. Eyes are closed.

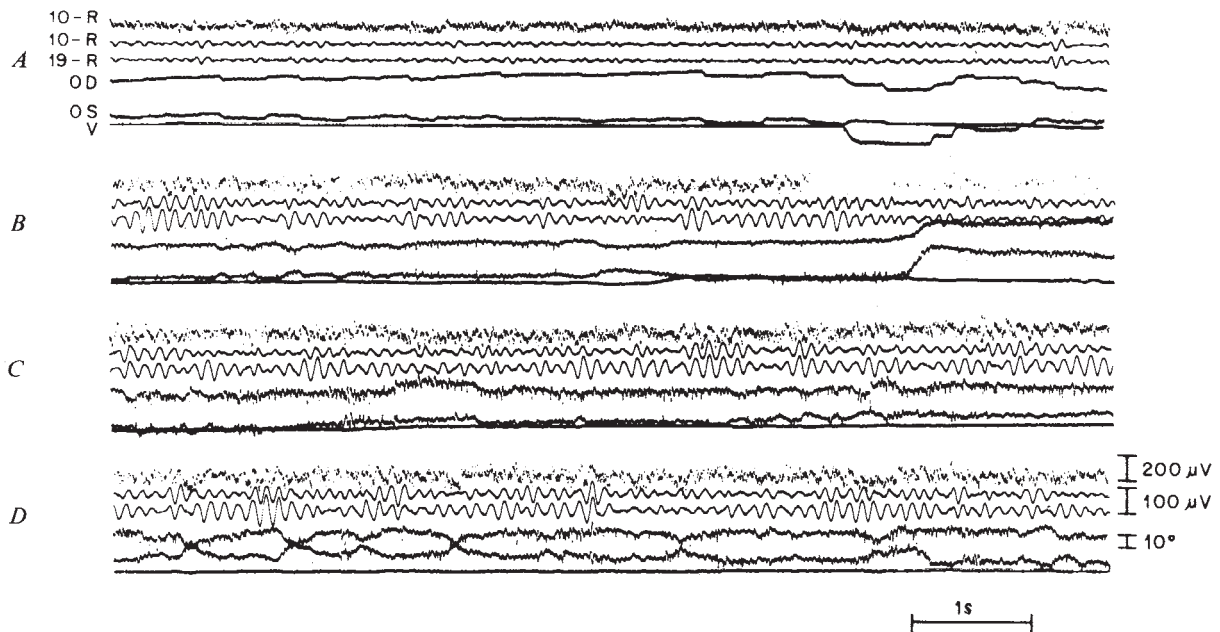


Fig. 2 A, Subject reading a newly presented passage from the *New Yorker* and trying to build up eidetic image. Note absence of alpha rhythm. B, During this section subject's eyes were closed and she read aloud the page eidetically built up in A. C and D, Subject had previously built up an eidetic image of a 61 $\times$ 68 cm print of Chagall's "My Village" and is describing aloud its details. Note the presence of frequent large amplitude alpha rhythm. Note the brief convergent eye movements in D.

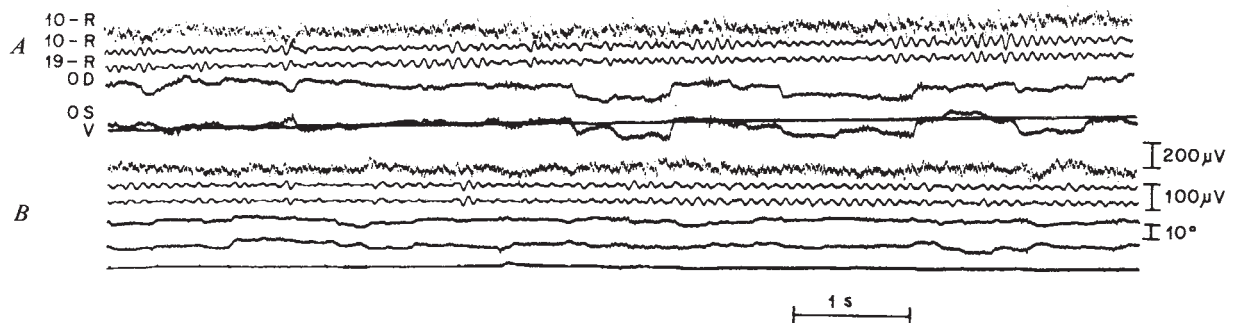


Fig. 3 A, The subject's eyes are open, and she has been asked to "place" an eidetic image of a page from Goethe's *Faust* at 6 m and read it aloud. There is a gradual build-up of alpha rhythm of low amplitude. (Compare with Fig. 1C, where subject eidetically reads a page from *Faust* with her eyes closed.) B, Subject has been asked to place eidetic image on a blank page 25 cm away from eyes and eidetically read it aloud. There is still some build-up of alpha rhythm but the amplitude is relatively low.

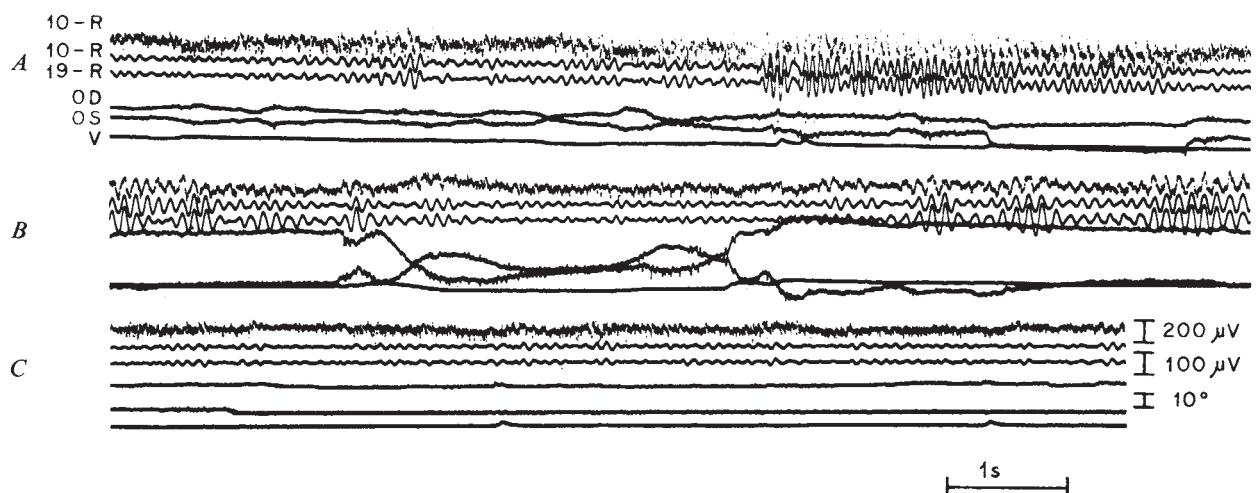


Fig. 4 A, Subject has been asked to bring in an eidetic image of Chagall's "My Village" as close to her nose as possible. Following relaxation of convergence there is an explosive release of rhythmic activity in the EEG at 16 Hz gradually slowing down to alpha frequency. B, Another series showing voluntary convergence as subject attempts to bring an eidetic image close. Note increase of alpha rhythm amplitude with relaxation of convergence. C, During active build-up of an eidetic image of a 66 $\times$ 46 cm print of Corot's "Pont au Change" there is virtually a complete block of alpha rhythm.

of convergence large amplitude rhythmic waves at 16/s appeared in the EEG and these gradually slowed and decreased in amplitude (Fig. 4A). When she examined an eidetic image during forced convergence her background alpha rhythm was markedly attenuated and then rebounded when convergence was relaxed⁹. During vigorous convergence the subject still had a single eidetic image.

Two types of eye movement were observed during recall of eidetic images. The usual movements were small saccades (Fig. 2A) which were not identical to those made during the build-up period (Fig. 2B). The subject eidetically read the same passage from *Faust* on two separate occasions; no consistently similar saccadic sequences were observed. Sequences of convergence were recorded when the subject described additional details of the Chagall print (Fig. 2D). On one occasion the EOG traces suddenly dipped down indicating an eye movement to the lower left. The subject reported that an eidetic page had "slipped off" to the lower left.

The findings demonstrate that this subject who possessed remarkable eidetic imagery had a normal alpha rhythm when she sat relaxed with her eyes closed and that this rhythm blocked when she opened her eyes and/or did tasks demanding a high degree of visual attentiveness. In these respects the presence and behaviour of her alpha rhythm are no different from those of other normal subjects. The presence of a large amplitude alpha rhythm from occipital leads when the subject read an eidetic page or scanned an eidetic painting either to herself or aloud is of much interest. These tasks required and received considerable concentration and mental effort as shown by the speed and detail of her recall. In other studies⁸ we have shown that it is that aspect of visual attentiveness or mental effort which requires resolution of or a search for the finest detail rather than the fine detail itself which blocks the alpha rhythm. Thus, the presence of alpha rhythm during eidetic recall is understandable if the subject either does not require the finest details for eidetic recall (as perhaps suggested by her description of the Chagall print) or alternatively that her access to fine detail does not require much search of the memory.

One may wonder whether the eidetic process is qualitatively or quantitatively different from visual memory recall in general. Presumably "gross" memory could involve recall of coarser detail with memory improving as finer detail is recalled. Recent work indicates that the striate cortex transforms the topographic representation of visual space into a Fourier transform or spatial frequency representation for restricted regions of visual space¹⁰⁻¹². In terms of Fourier theory fine detail is represented by high spatial frequency information and coarser detail by lower frequencies. The detail an eidetiker achieves might involve recall of a wider range or greater "strength" of spatial frequencies than is available to the non-eidetic.

Finally, we note that saccadic movements made during eidetic recall might serve to impart some spatial sense of "where" the image is, as the relative position or "distance" of an eidetic image depended on the position of the eyes.

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Frequency Response of Bat Tympanic Membrane

VESPERTILIONID bats produce FM echolocation pulses which sweep through a broad ultrasonic range^{1,2}. Neurophysiological^{3,4} and behavioural⁵ evidence indicates that *Myotis lucifugus*, the most intensively studied of the vespertilionids, is maximally sensitive to the frequencies of its cries. The small size and mass of the middle ear structures in Microchiroptera⁶ suggest that the transduction system is specialized for the reception of ultrasound. It is obvious that the bat middle ear must respond to high ultrasonic frequencies, but it is not known how efficient the middle ear is, or whether this is achieved by tuned resonances or by a broad-band response. We have used the Mössbauer technique to investigate the contribution of the tympanic membrane to this high-frequency sensitivity. Although our primary interest was in the high-frequency response, we examined a broad range from 1 to 100 kHz.

Five male specimens of *Eptesicus pumilis* were captured while roosting in a cave in the Meekatharra region of Western Australia. They were anaesthetized with 'Nembutal' (45 mg kg⁻¹) and mounted on a small platform in a manner similar to that described by Grinnell³. The tragus was removed and the pinna was split and reflected to provide an unobstructed view of the eardrum. In some cases the pinna and meatus were removed in order to expose the drum completely. The effects of opening the bulla were also investigated.

The velocity of the eardrum at frequencies up to 100 kHz was measured using the Mössbauer effect. This technique has been described in detail elsewhere^{7,8}. In summary, a 10 × 100 × 200 μm source of ⁵⁷Co diffused into palladium foil was placed at the apex of the concave eardrum, adjacent to the manubrium of the malleus. The gamma rays emitted by the source were counted through a stainless steel absorber, and sound pressure level (SPL) was adjusted until the count rate with sound on was 5-45% greater than that with sound off. The sound stimulus was gated to produce a 7 s on, 7 s off cycle and counts were accumulated for approximately eight cycles. The percentage count differences were transformed to velocity and converted to velocity and amplitude at 100 dB SPL (relative to 0.0002 dyne cm⁻²).

Free-field stimulation was used, with a high-power driver for low frequencies and an electrostatic speaker for frequencies above 50 kHz. The speaker was positioned 7-8 cm from the eardrum and SPL measured by means of a 3 mm condenser microphone (Bruel and Kjaer type 4138) positioned adjacent to the drum. The differentiated output of the condenser microphone was monitored and only frequencies at which the waveform was undistorted were used. Sound intensities normally varied from 86 dB at 28 kHz to 110 dB at 100 kHz. After some experiments intensity measurements were further checked by placing a second 3-mm microphone at the position of the drum. Some animals were run both at room temperature (24° C) and at 35° C. For comparison purposes, three guinea-

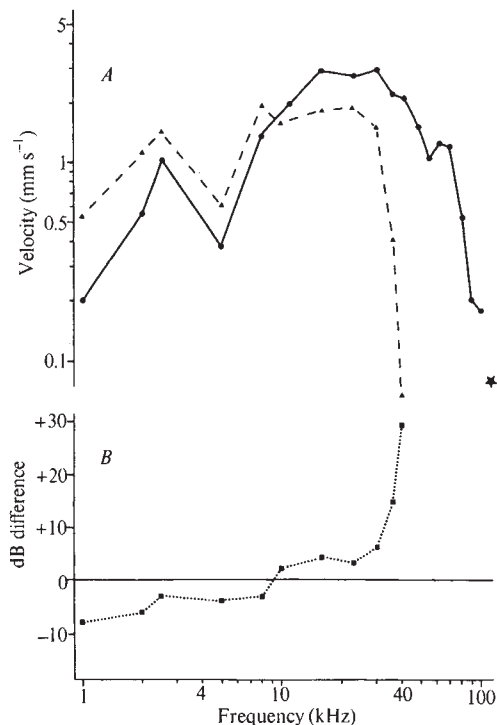


Fig. 1 *A*, Mean eardrum velocities for bat and guinea-pig at 100 dB SPL. Frequency scale as in *B*. Standard errors ranged from 0.4 dB (at 23 kHz) to 2.8 dB (at 1 kHz). Star indicates velocity at 115 kHz in one bat. Δ --- Δ , Guinea-pig; $\bullet$ --- $\bullet$, bat. *B*, Velocity difference in dB between bat and guinea-pig eardrums. Positive values indicate greater sensitivity in the bat.

pigs were tested in the same conditions. In these animals the drum was exposed by removing all of the cartilaginous and most of the bony meatus. The orientation of the speaker, microphone and counter tube relative to the drum closely approximated that used for the bat.

The mean velocity curves for bat and guinea-pig at 100 dB SPL are presented in Fig. 1*A*. The bat curve is based on five ears for which data were obtained at the same frequencies; a further three ears for which less complete data were obtained exhibited very similar responses. The most striking feature of these curves is the high-frequency superiority of the bat tympanic membrane. The guinea-pig response falls off rapidly above 30 kHz and could not be measured above 40 kHz. In contrast the bat response is maximal from 8 to 70 kHz, beyond which it also falls off rapidly. The high-frequency fall-off in the bat is approximately 40 dB/octave; in the guinea-pig 60 dB/octave. All models characterize the high-frequency response of the middle ear as mass-limited, with a 6 dB velocity (12 dB amplitude) fall-off. These data are the first available quantitative results on the high-frequency response and indicate that the mass-limiting dominates only up to a certain frequency. Beyond this, the fall-off is very rapid and must be due to more complex phenomena.

In Fig. 1*B* bat membrane velocity is expressed in dB relative to that of the guinea-pig. The bat membrane is less sensitive below 8 kHz. Above this frequency it is more sensitive and its relative advantage increases to a maximum of 29 dB at the highest frequency at which the guinea-pig response could be measured. The same dB differences would, of course, be obtained from a graph of amplitudes. Maximum mean amplitude for the bat tympanum at 100 dB SPL was 0.0656 μ m at 2.5 kHz and declined to 0.00029 μ m at 100 kHz.

Both curves in Fig. 1*A* show a notch in the region of 4–5 kHz. Others have observed a corresponding peak in guinea-pig eardrum impedance measures in this frequency range and have attributed it to cavity resonance in the middle ear^{10,11}. In our experiments small openings in the bulla shielded from the sound source (approximately 3 mm² in guinea-pig and

1.5 mm² in the bat) had no systematic effect on the size or the location of the notch. There were no significant differences between the responses obtained at different temperatures. We obtained similar results to those reported here from a single specimen of *Nyctophilus geoffroyi*.

These data indicate that the middle ear of *Eptesicus pumilis* is specialized for the reception of ultrasound. An important factor in this specialization seems to be the small area of the drum in bats⁶. We found a mean area in *E. pumilis* of 2.1 mm²; the area in guinea-pigs is 25 mm² (ref. 12). It is not clear to what extent ossicular and inner ear characteristics affect the response measured at the tympanic membrane. Relative to the guinea-pig, this high-frequency sensitivity seems to have been achieved with only minimal loss of low-frequency response. Although the vocalizations of this species have not been analysed, the region of maximal sensitivity corresponds well to the frequency range utilized by other vespertilionids.

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Natural Selection for a Quantitative Character over Several Generations

THERE are many plausible models to describe how selection might affect the values of a quantitative character. Perhaps the most generally useful is a quadratic model in which fitness decreases in proportion to the square of the deviation of the character from an optimum value. If w is the fitness of an organism when the character has the value x , then $w = 1 - \alpha - K(\theta - x)^2$ where θ is the optimum value of the character and K is a constant. Thus at $x = \theta$, $w = 1 - \alpha$ and fitness is at a maximum. From this model the values of $\Delta\bar{x}$ and ΔV_x , the changes in the mean and variance after selection, can be predicted¹. Conversely, if $\Delta\bar{x}$ and ΔV_x are known, then the values of the parameters, α , K and θ , and the change in mean fitness can be calculated². The proportionate change in mean

fitness, $\Delta\bar{w}/\bar{w}$, is a general measure of the effect of selection. While selection is acting only on phenotypes and there has been no mating, $\Delta\bar{w}/\bar{w} = V_w/\bar{w}^2$. But if sexual reproduction takes place after the selection, the formulae for $\Delta\bar{x}$, ΔV_x and $\Delta\bar{w}/\bar{w}$ may no longer be valid. Changes that affect environmental variations will not be passed on to the next generation: changes in the numbers of genotypes will be subject to genetic segregation and recombination. The variance may increase or decrease depending on the frequencies of the genes. In this communication, formulae are derived for the changes in the mean and variance of a character after selection from one generation to the next. They depend on a simple model of the inheritance of the character.

If the genes have only additive effects on the character—if, for example, the genotypes AA , Aa and aa have mean values $m+a$, m and $m-a$ —then the heritability, $h^2 = V_G/V_x$, can be used to calculate the change in the mean after reproduction. In this equation V_G is the variance in the genotypic values and V_x is the phenotypic variance. If $\Delta\bar{x}$ is the phenotypic change, then in the next generation the genotypic change will be $\Delta\bar{x}_G = h^2\Delta\bar{x}$. Unfortunately, the heritability cannot be used to predict the variance of the next generation. The variance may increase if the rarer alleles are increasing in frequency or may decrease if the commoner alleles are increasing. But the sign of the third moment (or, more accurately, the third cumulant) depends on the gene frequency and can be used to determine the changes in the variance.

For n loci with additive genetic effects on the character, the genotypic mean and variance are given by

$$\bar{x}_G = m + na(p - q)$$

$$V_G = 2na^2pq$$

where p and q are the frequencies of the alleles A and a ($p + q = 1$). By taking the first difference of these expressions, we get the equations

$$\Delta\bar{x}_G = 2na(\Delta p)$$

$$\Delta V_G = 2na^2(\Delta p)(q - p) - 2na^2(\Delta p)^2$$

If the term in $(\Delta p)^2$ in the expression for the change of variance is negligible, then since the third moment of the distribution is $\mu_{3G} = 2na^3pq(q - p)$

$$\frac{\Delta\bar{x}_G}{\Delta V_G} = \frac{2na^2pq}{2na^3pq(q - p)} = \frac{V_G}{\mu_{3G}}$$

This equation is a particular case of a general one between the cumulants of the binomial distribution³. It is

$$\frac{d\kappa_r}{dp} = \frac{\kappa_{r+1}}{pq}$$

where κ_r and κ_{r+1} are the r th and $(r+1)$ th cumulants. This gives

$$\frac{d\kappa_r}{d\kappa_{r+1}} = \frac{\kappa_{r+1}}{\kappa_{r+2}}$$

Kimura⁴ showed that for genes having additive effects on fitness, the change of genetic variance in fitness is $\Delta V_{Gw} = \mu_{3w}$.

If environmental effects are independent of genetic effects, then in general $\kappa_r = \kappa_{rG} + \kappa_{rE}$, and in particular since $\kappa_3 = \mu_{3G} + \mu_{3E}$. If the distribution of environmental effects is symmetrical—it might be normal for example—then $\mu_{3E} = 0$. Since $\Delta\bar{x}_G = h^2\Delta\bar{x}$ and $V_G = h^2V_x$, we have

$$\Delta V_G = \mu_{3G}\Delta\bar{x}_G/V_G = \mu_{3G}\Delta\bar{x}/V_x$$

Consequently, if the heritability is known, the genetic changes from one generation to the next can be calculated. The third moment also changes, but more slowly because its rate of change depends on the fourth cumulant. To allow for this change we could calculate $\Delta\mu_{3x} = \kappa_{4x}\Delta V_G/\mu_{3x} = \kappa_{4x}\Delta\bar{x}/V_x$. Changes in the higher cumulants will generally be very slow

indeed. The likely magnitude of these changes, and hence the error, can be found from the values of κ_{4x} and κ_{5x} . The heritability will itself change as V_G changes. In the next generation the phenotypic variance is $V_x' = V_G + \Delta V_G + V_E$ where V_E is the environmental variance. Thus the heritability becomes $h'^2 = (V_G + \Delta V_G)/(V_x + \Delta V_G)$.

In the model of selection in which $w = 1 - a - K(\theta - x)^2$, it can be shown that¹

$$\Delta\bar{x} = \frac{2V_x(\theta - \bar{x}) - \mu_{3x}}{\phi - (\theta - \bar{x})^2 - V_x}$$

where $\phi = (1 - a)/K$. This model was fitted to some data⁵ on selection for breeding times in the Arctic skua. The breeding periods into which the data are classified are periods of 7 days starting from June 10. The date of the hatching of the first egg was used as the breeding date. Data of pairs laying two eggs are given in Table 1.

Table 1 Relative Fitness of Skua Parents

Breeding period	No. of pairs	No. of chicks fledged	Relative fitness observed	Relative fitness predicted by the quadratic model
0	20	32	0.95	0.99075
1	114	192	1.0	0.98857
2	61	92	0.89549	0.90393
3	23	28	0.72283	0.73683
4	3	3	0.59375	0.48726

The values of $\theta = 0.47354$ and $\phi = 24.254$ are obtained when the model is fitted to the data. (The details will be published elsewhere.) Thus we can calculate the successive changes in $\bar{x}$ and V_x , the mean and variance of breeding time. The initial statistics are $\bar{x} = 1.43439$, $V_x = 0.71954$ and $\mu_{3x} = 0.37428$. The results, assuming for simplicity that $h^2 = 1$, are shown in Table 2.

Table 2 Mean and Variance of Breeding Times

Generation	Mean	Variance
0	1.43439	0.71954
1	1.35668	0.67912
2	1.28764	0.64107
3	1.22585	0.60499
4	1.17020	0.57057

The example chosen to illustrate the use of the formulae is artificial because the heritability of the breeding time is not known; and it is well to remember the assumptions that have been made in deriving the formulae. In particular, the alleles that determine the character have been assumed to have only additive effects. The gene frequencies have been assumed to be equal at the loci, which is very unlikely. But a paper to be published later will show that if selection is directional so that at any locus i , $\Delta p_i = s_i p_i q_i$ where s_i is the selective coefficient, then the formula $\Delta V_G = \mu_{3x}\Delta\bar{x}/V_x$ holds true regardless of variations in the gene frequencies. This is because the expressions for ΔV_G and μ_{3x} both contain similar terms involving the variance in the gene frequencies between loci. But if selection is not simply directional, other dissimilar terms are involved.

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BOOK REVIEWS

Documenting Science

Isis Cumulative Bibliography: a Bibliography of the History of Science formed from Isis Critical Bibliographies 1-90, 1913-65. Vol. 1. Part 1. Personalities, A-J. Pp. lxxviii+664. Vol. 2. Part 1. Personalities K-Z. Part 2. Institutions. Pp. 789. Edited by Magda Whitrow. (Mansell Information: London, 1971.) (Published in conjunction with the History of Science Society.) £28; \$67.20 the set.

THESE two magnificent and invaluable volumes are the first fruits of a larger project. They also represent the culmination of the work and dreams of George Sarton (1884-1956), the pioneer of, and tireless propagandist for, the discipline of the history of science. These volumes, therefore, have historical significance, as well as immediate and enduring practical utility for everyone interested in any aspect of man's scientific achievement.

In the decade between 1910 and 1920, the young Belgian scholar George Sarton found and settled to his life-work. Sarton was convinced that "the history of science is the history of mankind's unity, of its sublime purpose, of its gradual redemption". He therefore sought to rescue that history from the casual attitude and sloppy scholarship it so readily attracted. His aim was to establish the history of science as an independent intellectual discipline. In keeping with his Utopian temperament and singleness of vision, he was soon launched on ambitious schemes. A scholarly journal was central to his plans. *Isis*, his "revue consacré à l'histoire de science", first appeared in 1912. A multi-volume *Introduction to the History of Science* was projected as the fundamental reference work. A more descriptive "Harvard History of Science" in eight illustrated volumes was also envisaged, together with an annual series of *Studies in the History and Method of Science*, an exhaustive catalogue of past scientific instruments, facsimile reproductions of great scientific texts, a "Chinese encyclopedia" and an exhibition of the progress of

science, to name only some of the elements which Sarton came to see as part of his grand design.

That Sarton was unable to fulfil these heroic visions is scarcely surprising. While *Isis* has gone from strength to strength (since 1924 as the organ of the American-based, but international, History of Science Society), the *Introduction* had only progressed to AD 1400 (if also to 3,740 pages) when he reluctantly abandoned it in 1948. Most of the other plans remained but plans. Yet Sarton provided not only the basic journal and a fundamental reference text. Through tireless example, exhortation and propaganda over the years, he was also largely responsible for establishing the concept of the history of science as a discipline, demanding at least the rigour and standards associated with natural science itself. And as an integral part of his crusade, he had from the very start insisted on the necessity of critical bibliography.

Today we enjoy the fruits of such pioneering work. The history of science has now reached a certain maturity, both as intellectual discipline and as academic profession. This may be seen from a cursory inspection of the literature. *Isis* (now in its sixtieth year) has been joined by perhaps two dozen other journals, and by specialist series like the *Harvard Monographs on the History of Science*. The American Council of Learned Societies is sponsoring a magnificent *Dictionary of Scientific Biography*, of which four volumes have already appeared. In England the very recent creation of an Oxford chair in the history of science gives recognition of the standing and significance of the discipline. And the publication of these first two massive volumes of the *Isis Cumulative Bibliography* provides a measure of how far our understanding of past science has grown, and how much there is still to achieve.

Sarton saw the provision of critical bibliography as central to his tasks. Every volume of *Isis* has included at least one such bibliography. In the early years they reflected both Sarton's

holistic desire to embrace all human knowledge, and his necessarily limited resources and information, as he ploughed his lonely pioneer furrow. Since then the bibliographies have become regular annual productions of more limited scope but more thorough coverage. Their significance as guides to the history of science is thus undisputed. However, any scholar concerned to identify the literature on a person or historical event has faced the daunting task of consulting an ever-growing pile of these guides, with their shifting and maturing indexes and analytic schema. By 1965 ninety such bibliographies were extant, and some consolidating action was urgently needed. Now, thanks to the heroic labours of Mrs Magda Whitrow, and the advice and support of an editorial committee under Professor I. Bernard Cohen, we have available an initial two volumes of the *Isis Cumulative Bibliography*.

The magnitude of the task that confronted Mrs Whitrow may be gauged by the application of a little Sartonian arithmetic. The *Cumulative Bibliography* draws on some 2,500 journals over a period of 52 years, and in these two volumes provides information on around 10,000 individuals and 1,750 institutions, in 1,462 quarto pages of main text. Further volumes now in preparation will analyse the material stored in the *Isis* bibliographies by civilization, period and subject. That material still bears some dim trace of Sarton's holistic ambitions. It ranges from the theory of knowledge to the history of medicine. The *Cumulative Bibliography* will thus be the one indispensable key to any understanding of the growth, nature and meaning over the ages of science as an intellectual and social enterprise, as reflected in scholarly enquiry and discussion through the troubled half-century from Sarajevo to Sputnik.

Because the early *Isis* bibliographies were highly idiosyncratic, and because the present compilation necessarily reflects its editor's criteria, any specialist will be able to point to shortcomings

in his own field of interest. However, the point about this cumulative bibliography is its amazing range and scope. Hard-won and half-buried knowledge is now properly indexed and quickly available, thanks in part to the publishing techniques developed by Mansell Information. Even with a brief perusal, one can begin to discern patterns only half-suspected in the way Western historians have reflected on scientific endeavour. Once again, a little Sartonian arithmetic makes the possibilities clear.

The *Cumulative Bibliography* provides entries for some 10,000 individuals, yet only 200 earn more than thirty entries: striking evidence for the central importance of great men to the progress of science, or to our mythology of science, or more probably to both. Aristotle takes the prize as most studied individual (*circa* 450 entries), leaving Plato (*circa* 270), Newton (*circa* 320) and Einstein (*circa* 120) far behind. Paracelsus receives 200 entries but Justus Liebig only fifty and Ernest Rutherford thirty-five.

The institutional section highlights our ignorance of the social fabric of science. The Royal Society completely dominates but can only manage 123 entries, forty-two of which are focused on the seventeenth century, three on the nineteenth and none on the twentieth! The French Académie des Sciences obtains a grand total of only twenty-seven scholarly studies, while the US National Academy of Science limps in with five. The Cavendish Laboratory succeeds in attracting twelve enquiries, while none of the laboratories at Manchester manages a mention. Obviously this *Cumulative Bibliography* provides both food for thought and endless scope for private browsing. Equally obviously it provides an indispensable research tool for every serious reference library. All associated with its production are to be heartily congratulated, and urged on to the completion of their labours.

ARNOLD THACKRAY

Membrane Biology

The Chemistry of the Cell Interface. Edited by Harry Darrow Brown. Part A. Pp. xi+341. (Academic: New York and London, October 1971.) \$22; £10.25.

The Chemistry of Biosurfaces. Edited by Michael L. Hair. Pp. xiii+376. (Marcel Dekker: New York, August 1971.) \$22.50.

BOOKS on various aspects of membrane structure and function are coming from the press thick and fast nowadays, so that readers can be selective about what they buy. Reviewers, in their turn, can demand something out of the ordinary:

originality, an especially clear, attractive, up-to-date presentation, or unusual value for money. *The Chemistry of the Cell Interface*, edited by Harry Darrow Brown, has no such marks of distinction. Costing £10 for 341 pages, it is not cheap. With all but a few references to work before 1970, it is not up-to-date in a field of study expanding logarithmically. There are relatively few illustrations, especially in half-tone, and all but two of the electron micrographs are on well known subjects and borrowed from non-contributors.

The book is nevertheless a useful summary of the state of knowledge early in 1970, with much information of permanent value carefully documented. A long chapter by Brown and Chattopadhyay on organelle-bound enzymes lists and discusses in detail enzymes associated with endoplasmic reticulum, Golgi apparatus, mitochondria, lysosomes, microbodies and plasma membranes—including transport proteins and adenyl cyclase. The section on enzymes of the smooth endoplasmic reticulum which are responsible for drug metabolism is especially comprehensive. The lists of enzymes associated with particles provide a convenient reference.

The most original chapter is by Laidler and Sundaram on the kinetics of supported enzyme systems. Although much useful information about enzyme kinetics has been obtained by studying reactions in solution, many enzymes in cells are not in free solution but attached to structural material, usually membranes. Their kinetic behaviour is different from that in free solution. Laidler and Sundaram discuss the kinetics of enzymes attached to solid phases of various types by physical or chemical links and compare these with the kinetics of membrane-associated enzymes.

The chapter on particles by Dieckert is sound but covers for the most part familiar ground. H. T. Tien and L. K. James, jun., consider in general terms lipid-lipid and lipid-protein interactions, including monolayers, micelles, soluble lipoproteins, bimolecular lipid membranes at an interface and between two fluid layers (the valuable model of Mueller and Rudin) and liposomes (the model of Bangham). There is little new material in this chapter, but the main advantages and limitations of each system are well set out. Natural membranes are briefly discussed by Tien and James. The same theme is repeated with minor variations in an introductory chapter on the chemistry of membranes by Van Bruggen. This chapter is distinguished mainly by the number of misspellings of authors' names (for example, Gortner for Gorter, van Deenan for van Deenen). It is remarkable that the interrupted bilayer model of membrane structure, which is now

so widely held, receives rather little discussion, and the concept of translational movement of membrane glycoproteins none at all. Both of these concepts are already transforming our views on membrane behaviour.

The Chemistry of Biosurfaces, which consists of a series of chapters from different contributors on the chemistry of the cell surface, enzymes at surfaces, growth of cells on different surfaces and related topics, is intended to bring together recent information on surface chemistry insofar as it has relevance to biological systems. Some of the technological advances, for example the use of synthetic polymers for artificial joints, blood vessels, dental prostheses and so on, raise problems of surface chemistry which have immediate application to biology and medicine. Other recent physico-chemical studies, which emphasize the complexity of even simple surfaces and their interactions with adsorbed materials, show that analogous phenomena in biological systems must be even more complex.

Some of the chapters are fairly detailed physico-chemical presentations which will be beyond the reach of all but a few research workers in the biological and medical fields. One of these is the chapter on hydrophobic bonding by Dandliker and de Saussure. Although this subject can be treated mathematically in a manner which is satisfactory for most practical purposes, it can only be understood by reference to the structure of water and the effects on it of insertion of nonpolar groups. Our knowledge of both of these basic problems is far from complete. Even the name, "hydrophobic bonding", although firmly entrenched, carries the wrong implications, as in the analogous case of the name "guinea-pig" for an animal that does not come from Guinea and is not a pig. Among the effects described by Dandliker and de Saussure are those of chaotropic ions, which increase disorder in water structure and break hydrophobic bonds. Their effects in biological systems have been insufficiently explored: for example, they can dissociate complexes of antigen and antibody at neutral pH.

The most generally interesting chapter is that by Tien on bilayer lipid membranes. This is one of the two most useful experimental models for biological membranes, having remarkable electrical properties closely resembling those of nerve and muscle membranes, as well as some properties of photoreceptors. The construction, physico-chemical properties and biological applications of such black membranes, as they are usually termed, are well covered. A straightforward account of the chemistry of lipids in water is contributed by Abramson, including physico-chemical studies of lipid mono-

layers and aqueous dispersions of lipids.

Three chapters are concerned with surface chemistry: adsorption of molecules which can be taken as analogues of biological molecules, onto non-biological surfaces (Fontana), adsorption of surfactants at solid-water interfaces (Fuersteran) and adsorption of proteins and lipids to non-biological surfaces (Brash and Lyman). Although all are well written, the choice of material seems arbitrary. It would have been possible, for example, to choose examples of surfactant action of considerable biological relevance; and the importance of hydrogen bonding in explaining the action of surfactants on living systems, as worked out by Nash and others, is not mentioned.

The final chapter on cellular narcosis and hydrophobic bonding by C. S. Hersh is more rigorous physico-chemically than Ferguson's well known thermodynamic approach to the problem. The presentation, however, still offers little information about which biological materials are likely to be important in anaesthesia (for example, lipid or protein of the membrane or some other site).

In general, the book is clearly written, well produced and illustrated, and accurate. Occasional misprints—for example, on page 285, solvents in black membranes are described as "space filters"—are of minor importance.

A. C. ALLISON

Electronic Theory

Thermodynamics of Electrical Processes. By Malcolm McChesney. Pp. ix+278. (Wiley: London and New York, 1971.) £4.25.

THE preface is quite clear about intentions: the book is written by a professional thermodynamicist for second-year, third-year and perhaps early postgraduate students of electrical engineering and physics. The systems of primary interest are metals and semiconductors, many of whose properties are explicable in terms of interacting gases of electrons and phonons in imperfect lattices. The author's view is that these matters are best understood against a background of statistical thermodynamics applied to perfect quantum gases. I find the approach most helpful and only wonder why the title is not more accurately descriptive. Personally I would have removed chapter 1 (thermodynamics revision) and the final chapter (on information theory, irrelevant to the main themes).

The formal statistical mechanics is conventional and restricted to the microcanonical distribution. The approach is well explained, with the aid

of examples and tables but missing out unduly sophisticated mathematical argument where a simple assertion will do. There is then a very detailed account of Maxwell-Boltzmann gases (including plasmas), Fermi-Dirac gases (including conduction electrons) and Bose-Einstein gases (including the phonon gas in solids). There follow 53 pages of excellent material on thermal and electrical transport in solids, and another well-presented 65 page chapter concentrating on band theory and electrons in semiconductors. The omission of a detailed model (such as Kronig-Penney) perhaps illustrates the slightly arbitrary criteria for selection of material in the book. Anyway I think it is fair to say that arguments are predominantly physical, based on the mathematics of the earlier "service" chapters or merely asserted.

The layout is pleasant and there are a few problems after each chapter.

DAVID BETTS

The Metallic State

Magnetic Resonance in Metals. By J. Winter. Pp. xiii+206. (Clarendon: Oxford; Oxford University: London, November 1971.) £5.50.

THIS monograph presents a uniform and coherent view of the relationship between the nuclear and electronic spin systems and the measurable quantities accessible to the experimentalist. The initial chapters provide a very brief introduction to nuclear magnetic resonance, the hyperfine interaction, and electrons in metals. These are the *sine qua non* of the subject at hand, and the coverage is adequate to introduce the bulk of the notation required subsequently. Chapter 4, entitled "Theoretical Study of the Nuclear Resonance Properties of Metals", promises to "include all the information about the metallic state that can be learned from a nuclear resonance experiment"—a statement designed to make a reviewer wince. Not surprisingly it is the longest of the ten chapters, and the treatment does introduce and develop essentially all of the basic concepts and many of the expressions found to be needed in the balance of the book. The theoretical treatment is concise, often invoking second quantization formalism. Were it not for the author's generally helpful physical arguments the coverage might be a bit too succinct in some places. The degree of theoretical sophistication of the reader, and also his motives in reading the book, will determine the adequacy of treatment. The unity and freshness of approach in the derivations is the strong virtue of the book and will be of undoubted help to the newcomer. The chapter (and book)

are admirable in presenting a clear and inclusive statement of the significant nuclear-electron interactions in metals.

Successive chapters deal with the application of the theory to pure metals, alloys, liquid metals, and liquid alloys. The presentation is smooth and appealing. At times liberties are taken with the number and placement of data points in the (apparently all redrawn) figures; however, these renditions are generally satisfactory for their primary purpose of illustrating the author's arguments. The referencing is confused in Fig. 7.3 and Table 7.1; the ordinates in Figs. 7.1 and 7.7 are wrong, and the abscissae are atomic %. Somehow these facts do not detract greatly, and are presented here in an attempt to convey a sense of the relative emphasis and attention given to presenting the experimental and theoretical aspects of the subject.

Transition metals (even as dilute additions to the noble metals) are treated separately with arguments stressing the role of their unfilled inner shells. The use of resonance to investigate suspected Kondo behaviour is described, including reference to the local spin fluctuation view. Space does not permit a properly appreciative review of the chapters on superconductors and spin resonance of conduction electrons. The latter especially is very nicely done. Its material is used by reference throughout the book and enhances the entire effort.

Experimental technique is not discussed; some areas (for example motional effects), perhaps of less interest to some physicists, are essentially omitted and reference to them is annoyingly weak. Also, more frequent use of the 204 references given (ending in mid-1969) would have been beneficial. No tabulated reference data are included. The value of a Knight shift for a metal must be found elsewhere. Author and subject indexes are present and adequate; misprints and errors are few and not serious. The reader would be wise to refer to the original paper of Kohn and Vosko (Winter's ref. 95) for a more lucid derivation and numerically correct expression of Equation (7.25).

T. J. ROWLAND

Journal on Environment

Environmental Physiology: Nutrition, Pollution and Toxicology. Edited by J. Clausen. Associate editors, A. C. Allison, J. Ganguly, W. Staib, E. M. Boyd, B. Holma and P. S. Timiras. Vol. 1. No. 1. Pp. 54. Vol. 1. No. 2. Pp. 55-103. (Munksgaard: Copenhagen, 1971.)

THE stated scope of this new journal "encompasses experimental work on all environmental factors of a physical, chemical and/or physico-chemical

nature, which have influence on living organisms, especially the mammalian body".

There is a growing and not unnatural tendency to greet the creation of any new journal with some resentment at further encroachment on one's time and wallet and, more directly, with a challenge as to its *raison d'être*. *Environmental Physiology* assuages many of these feelings. Problems of environmental pollution and environmental toxicology are considered from the broadest possible standpoint of physiological perturbations and are thus rescued from restricted approaches of classical toxicology. The composition of the editorial and advisory boards has a strong international flavour, with the exception of the absence of any Japanese representation. The journal seems to be free from the heavy industrial domination and implicit constraints that characterize the established American and British toxicological journals.

The scientific quality and interest of the thirteen articles in the first two numbers of this journal are generally of a high order. A broad range of subjects is dealt with, including depression of proximal renal tubular function by methylmercury in the rat, placental transfer and foetotoxicity of polychlorinated biphenyls, the effects of aflatoxin on nucleic acid and protein synthesis in the perfused regenerating rat liver, the influence of weather on respiratory tract fluid, and the effects of altitude on myocardial lipids of animals indigenous to various altitudes, on gluconeogenesis in mice, and on maturation of the central nervous system in the chick embryo.

The two-column-page format makes for easy reading and the overall design is attractive. Articles are concise, averaging about six pages, and reflect careful and uniform editorial handling. The promise of rapid publication of articles by the paper is welcome, but should be evidenced by the customary statements of date of receipt.

This journal has got off to a good start. It is attempting to help fill in the void in the critical field of environmental concerns and for these reasons merits the widest possible interest and support.

SAMUEL S. EPSTEIN

Fungitoxicity

Chemistry of Fungicidal Action. By R. J. Lukens. Pp. xiii+136. (Chapman and Hall: London; Springer: Berlin and New York, 1971.) £5.

AN attempt has been made in this book to cover all aspects of fungicidal activity and "Fungicidal Action" would per-

haps have been a more appropriate title. The treatment is logical enough. After a chapter on measurement of fungitoxicity, consideration is given to the barriers which the fungicidal molecule must overcome in order to reach its site of action. Then there are a number of more chemical chapters dealing with sites of fungicidal action and the various mechanisms which might operate in fungitoxicity at cell level. Specific groups of fungicides are selected for consideration of structure/activity relationships and physical and biochemical factors by which the fungus might influence the fungicide are also considered. A special chapter is devoted to the effects of fungicides on enzymes. Then follows an appendix listing the more important fungicides and their uses.

A criticism of this book is that recent and other important work on fungicides is not referred to either in the text or in the list of references. Furthermore, it is surprising that the subject of systemic fungicides, which is developing rapidly at the present time, is barely mentioned in the text. Nevertheless, the volume provides useful reading for research workers and students taking postgraduate courses in this field.

There are a few errors in chemical formulae and the book is expensive but it is well produced and is useful in that it brings together in one volume many of the factors which can operate in fungicidal action.

R. L. WAIN

Organotins

Organotin Compounds. Edited by Albert K. Sawyer. Vol. 1. Pp. xiv+252. Vol. 2. Pp. xiv+253-623. (Marcel Dekker: New York, 1971.) \$30 each.

THESE are the first two volumes of a three volume compendium on organotin compounds. Approximately twenty authors have contributed chapters on various aspects of the subject.

That organotin chemistry is now an extensive subject is apparent from the appearance of three major works on the topic in recent years. Neumann's monograph appeared in 1967, followed by Poller's work in 1970, with the volumes under review appearing in 1971.

Although the contributors have given excellent accounts of their subjects, and these two volumes have almost three thousand five hundred references to the scientific literature, there is a worrying variation in the recency of the literature coverage. Thus, for example, in chapters 4 and 5, references to post-1968 literature are non-existent, while chapters 6 and 7 have about 10 per cent of their references after 1968. From the

way authors have had to "insert" references, there are indications of a serious production delay in these books.

Quite recent articles on many of the individual chapter topics are available in the review literature of chemistry (often by the same authors). Nevertheless, this collection of reviews does represent the most complete and up-to-date coverage of the field of organotin chemistry that is available, and provided the indices in Volume 3 are adequate it will provide an important work of reference for non-specialists who want rapid access to certain aspects of organotin chemistry. Organometallic chemists would do well to read at least some of the chapters, to gain a realization of the truly remarkable range of reactions that now come within the sphere of organotin chemistry.

While we have come to expect a steadily increasing price range in scientific monographs, it is salutary to note that the average (per page) price of these volumes is double that of the comparable books by Neumann and by Poller! The nuances of the economics of scientific book production never cease to fascinate me. The "thinner" (Volume 2) of these two volumes contains over one hundred more pages than the "fatter" (Volume 1); yet both are priced at a rather steep \$30 each.

EDWARD ABEL

Thin Films in Detail

Thin Films. By K. D. Leaver and B. N. Chapman. Pp. vii+112. (Wykeham: London and Winchester, December 1971.) £1.50.

A LIST of examples of the way in which thin films have contributed to modern technology would be quite long. Processes which only a few years ago were confined to the research back-room have now become commercially commonplace and have completely transformed many industrial processes, notably in the electronics field. The subject of thin films is therefore a natural one for the Wykeham Science series, which aims at introducing areas of current interest to students approaching science courses. The authors of this book are well known for their many contributions to the field and have covered a large number of topics of importance and interest in the field. The material is clearly presented, if in a rather un lively style. In places, the amount of detail given seems excessive for the type of reader for whom the book is intended, and a concluding chapter putting the subject in perspective would have been welcome. Nevertheless the book does provide a useful range of information not otherwise accessible in this form.

O. S. HEAVENS

CORRESPONDENCE

Heads in Bags

SIR,—It is sad to read a scientist arguing unscientifically while he accuses another of doing the same (*Nature*, **236**, 43; 1972). The scientific approach to any problem usually includes firstly the attempt to be impartial and objective when analysing often conflicting data; secondly the endeavour to identify and measure cause-effect relationships; thirdly in arguing from the general to the particular rather than *vice versa*. Let Mr Fakes answer three questions which spring from his evident failure to follow such principles: "the justice of overthrowing tyranny by force is . . . honourable."

(1) Is the present, reformed Ulster constitution a "tyranny" or a democracy? Compare it with other constitutions. (2) Is that "force" considered "honourable" which involves the indiscriminate, avoidable, triumphant, planned killing and maiming of men, women and children? Contrast the quantifiable results of Mafia, Klu-Klux-Klan and IRA activities. (3) How often within modern history have the practitioners of violent revolution and the associated civil warfare produced a government superior to the "tyranny" which activated their violence? Calculate the ratio of their successes to failures, comparing it with a ratio similarly derived for the exponents of constitutional reform.

Further search for political science content in Mr Fakes's emotional conclusions becomes an exercise in futility when his implicit denial of the principle of impartiality in politics—especially Irish—is used to launch a denunciation of only one side's record. As a Celt oscillating between Pantheism and agnosticism I myself have really no idea where my bias here lies. However, it is the "prejudice" of most people including Irish Republicans that the vast majority of recent atrocities have IRA origins; as yet the "Protestant backlash" has not been implicated. That reciprocating bestialities were perpetrated in the past "recent history of Ireland" is well known but irrelevant to analysing the present bloodletting, unless the atavistic principle of blood feud is invoked.

It is a sad truism that the society which wishes to survive abandons its loftier legal principles to a degree and for a duration commensurate with the particular threat to its survival. In such situations leaders ignore their sources

of military intelligence with great cost to their subjects—Raglan in the Crimea, Chamberlain at Munich, Stalin's Russia 1941, Nasser's Egypt 1967, our Ulster 1972. In 1972 only a person living in a cloister could believe that moral narcissism deters the aggressor. As a contribution to political problem-solving Mr Fakes's amalgam of cloudy pontification, personal value-judgment, blatantly distorted history and political nihilism is inadequate.

Yours faithfully,

O. LL. LLOYD

8 Suffolk Road,
Edinburgh 9

Whither Physicists?

SIR,—I have read with interest your editorial on "Are there jobs for physicists to do?"—and the article entitled "Where will they go?" (*Nature*, **236**, 131 and 134; 1972).

I should like to add our experience, which is not as negative as that of Dr Swift-Hook. We believe, and have experience, that good physicists, with good class degrees, and possibly with PhDs, are very useful in both research and other activities in the company. We also think that the PhD itself does not add much to a man's suitability for industrial employment except perhaps in some branches of research. Even in research we find that people of equal ages are on the same average salary lines whether they have obtained a PhD or have come directly after their first degree. We also think that the university training in physics is in general a good training, and I would not like to see it much diluted.

However, we believe universities do not give students a sufficiently clear idea as to what they will do or what they will be able to do after graduating. Most physics graduates until a very few years ago could only envisage a career in research, and it is only recently that necessity has forced them to look at other jobs.

I am not certain whether the numbers of graduates in physics coming out of universities at the moment is too great for them to be absorbed in jobs requiring a knowledge of science and especially of physics. I think it probably is the case, but I have no evidence that a man who has been trained in physics is any worse at a job

in engineering or administration in industry than a man trained at university in another subject such as arts or social sciences.

Yours faithfully,

K. HOSELITZ

Mullard Research Laboratories,
Redhill, Surrey

Expert Committees

SIR,—I share the disappointment of your reviewer (*Nature*, **236**, 1; 1972) over the latest report of the FAO/WHO Expert Committee on Nutrition. Vast sums of money are spent on such UN agency reports and they do not merit the attention frequently given to them. The choice of invitees is political, rather than on the basis of scientific merit, and strongly influenced by the whims of the UN agency secretariat. When a group of virtual strangers is brought together with very little preparation for only a few days, it is too much to expect that a thorough study of the topics will result. It is usually left to the secretariat, with one or two of their friends, to write the final report. It is hardly surprising, therefore, that such reports when read serially show many inconsistencies, prejudices and glaring omissions. While the nutrition units of FAO and WHO were temporizing with "protein-rich" weaning foods, lysine fortification, nutrition rehabilitation centres, and overemphasizing the "protein gap" and kwashiorkor, private foundations were making the only significant contribution to solving the malnutrition problem of our time: the green revolution.

A fresh, hard look should be taken by the UN at its expert groups and their reports. In Britain and some other countries technical committees spend months, and if necessary a year or two, to produce reports which their governments feel they can set their seal to as representing a balanced account of the subject at that time. It is high time for the UN agencies to drop their cavalier attitude towards their responsibility of acting as spokesmen for the world on matters of practical scientific importance.

Yours faithfully,

DONALD S. McLAREN

School of Medicine,
American University of Beirut,
Beirut

Obituary

Dr A. S. Russell



[Bassano and Vandyk]

WITH the death in Oxford on March 15 of Dr A. S. Russell, MC, for many years tutor in chemistry at Christ Church, another link with the great days of Rutherford and the beginnings of nuclear physics has been broken. His important contributions to the chemistry of the radio elements which led to the concept of isotopes are now part of the history of the subject.

Alexander Smith Russell, the second son of Mr John Russell, of HM Inland Revenue Department, was born on May 31, 1888, at the small fishing town of Musselburgh near Edinburgh. He was educated in Glasgow at the High School and then went on to the University where he obtained his MA in Mathematics and Natural Philosophy, and his BSc in Chemistry. In 1908 he commenced his research in radioactivity at Glasgow University under the direction of Mr Soddy (later Professor and Nobel Laureate in Chemistry): this early work was on gamma radiation from the radio elements and at the time this constituted the most exhaustive study so far made. He then went to Berlin for a year where he gained research experience working with Professors Nernst and Marckwald on specific heat determinations and on the radioactive content of uranium minerals. On his return to England in 1911 he moved to the laboratory of Sir Ernest Rutherford (later Lord Rutherford) at Manchester where he resumed his research on the chemistry of the radio-

active elements. It was during this period that he made some particularly significant contributions to the development of what came to be known as the "Displacement Law" and which led to the hypothesis and discovery of isotopes.

The Americans McCoy and Ross in 1907, followed by many others, had discovered pairs of radio elements which were physically and chemically inseparable. Now the rare earth elements had already presented chemists with great difficulties in their separation but these radioactive "inseparable pairs" seemed to fall into a different class of difficulty. In 1910 Soddy who like the others had worked with purely chemical techniques suggested that the members of these pairs were "not mere analogies but chemical identities". A year later on rather slender evidence Soddy pointed out that alpha decay seemed to displace the chemical properties to the next group but one in the periodic classification. It was at this stage that Russell and Rossi reported that the emission spectrum of ionium was identical with that of thorium and concluded their paper with the significant statement "... but the possibility that thorium and ionium are identical in all physical and chemical properties and differ only in atomic weight and radioactive properties should not be lost sight of". At about this time Russell pointed out to Soddy the likelihood that radiogenic lead would have a different atomic weight from non-radiogenic lead, a fact subsequently established by Soddy and regarded by him as convincing proof of the isotope theory as applied to heavy elements. In January 1913 Russell published in *Chemical News* the first clear statement of the Displacement Law in which he showed that alpha decay displaced the chemical properties two places and beta decay one place in the periodic table. With this he was able to construct the radioactive decay chains and he forecast the existence between U_I and U_{II} of two beta emitters which were subsequently identified. Two weeks later Soddy spelled out the Displacement Law more correctly and acknowledged his debt to Russell for his earlier formulation. At the same time Fajans quite independently reached the same conclusions and published a complete statement of the law. Later that year Soddy coined the word "isotope" to indicate that these "inseparable pairs" lay in

the same place in the periodic table.

In the following year war broke out and these exciting researches which are recorded in some 25 papers came to an abrupt end. Russell, still only 26 years old, joined the army and was commissioned in the Royal Garrison Artillery. He spent four years in the army, much of it in France engaged on sound ranging work at the front, and rose to the rank of captain. He was wounded twice, received several mentions in despatches and was awarded the Military Cross. On demobilization he became Senior Lecturer in Chemistry at Sheffield University and a year later was appointed Dr Lee's Reader in Chemistry at Christ Church where he remained until he retired in 1955.

During this post-war period he wrote two books and some 40 papers on his research, all marked by a beautiful succinctness and clarity which make them readily intelligible even to those unfamiliar with the field. Apart from his research in radiochemistry he was also interested in the occurrence of stable isotopes and he wrote several papers on empirical mathematical relationships which he concluded existed between the mass of a stable isotope and its atomic number. His major interest in these years, however, lay in metal chemistry, and he and his research students made an extensive study on the chemical stability and electrochemistry of inter-metallic compounds and amalgams.

His years as a college tutor gradually drew him into administration and in 1938 he was elected Censor of Christ Church, an office which he held throughout the Second World War and which brought him into touch with large numbers of undergraduates: his great interest in people and in their problems, and his wise guidance, earned their respect and support. When he relinquished office the members of the Junior Common Room presented him with a gold watch: such an unusual step showed with what affection he was regarded. His remarkable memory for facts, figures and personal histories was a valuable aid to his natural administrative ability. As a raconteur he was exceptional and his sense of humour, taste for the ridiculous and love of biographical detail made his stories riotously funny. Many had a strong scientific flavour and his acknowledgment of the genius of the Almighty for

having invented the Moon as a device for keeping our beaches clean made even his clerical colleagues smile.

Dr Russell belonged to that group of Oxford chemistry tutors of the inter-war years which was responsible for making the school internationally famous for the graduates which it produced. His hundreds of ex-pupils, all loyal and many distinguished, are as much a memorial to him as the contributions to knowledge which he made and which should be better known than perhaps they are.

Professor Maria Goeppert-Mayer

THE recent death of Maria Goeppert-Mayer is a cause for sorrow and regret to all who knew her and marks the end of a chapter in the history of nuclear physics.

Maria Goeppert was born in 1906 in Kattowice, then in Germany, and gained her PhD at the University of Göttingen, Germany, in 1930. In the same year she married Joseph Mayer and moved to Johns Hopkins University, Baltimore, with her husband. In 1933 she took out United States citizenship. She moved to Columbia University, New York, in 1939, becoming a senior physicist at Argonne National Laboratory and professor at the Enrico Fermi Institute of Nuclear Studies, Chicago, from 1946–1960. Since 1960 she and her husband have held chairs at La Jolla, California.

At Chicago she came under the influence of Enrico Fermi who suggested that she should see whether certain minor irregularities in nuclear systematics could be explained in terms of a model of the nucleus bearing certain similarities to the quantum mechanical model of the atom with its shells of tightly bound electrons. Thus was born the nuclear shell model which was to prove so fruitful for all later developments in nuclear theory. Previously it was thought that the short range and the extreme strength of nuclear forces would make a detailed description of the nucleus exceedingly difficult; indeed it was likened to a liquid drop in which the forces between the molecules have a similar character. It was not until the shell model had been well established empirically that it was realized that nuclear forces within the nucleus could not exert themselves as strongly as in empty space owing to the operation of the Pauli exclusion principle which, by limiting the states available to interacting particles to those not already occupied by other particles, effectively reduced their possibility for interaction. This is how it comes about that in spite of strong nuclear interactions the neutrons and protons within

the nucleus behave in first approximation as if they are weakly interacting—as do the electrons in the atom.

And so for the first time it became possible to develop detailed explanations of the spectra and other properties of nuclei. Although since those early days it has been realized that in addition to their near-independent particle motions nuclei also exhibit collective motions reminiscent of those originally imagined for a liquid drop nucleus, so that a nuclear spectrum resembles less that of an atom than a molecule (with its nearly independent electronic motions coupled to collective nuclear motions), the shell model remains the starting point for any meaningful considerations of nuclear structure.

It often happens in science that discoveries are made independently by more than one person or group. So it was with the nuclear shell model, for quite independently but simultaneously Hans Jensen and his colleagues in Heidelberg came to essentially the same conclusions. To add to the coincidence, Mayer and Jensen shared the same birthday. She and Jensen became close friends and colleagues and published a definitive account of their work in *Elementary Theory of Nuclear Shell Structure* in 1955. In 1963 her work was recognized by the award of the Nobel prize for physics, shared with Jensen and Wigner.

Mrs Mayer's early work was in statistical mechanics on which she worked jointly with her husband. Even after the discovery of the shell model, however, they collaborated closely. Indeed, the news of the shell model was brought to England by her husband in a lecture at the Clarendon Laboratory, Oxford. Their household was legendary for its hospitality, and their kindness and encouragement to young scientists knew no bounds even after the severe stroke which afflicted Mrs Mayer in her later years.

Announcements

Miscellaneous

Dr A. C. Allison, Clinical Research Centre, Harrow, will receive a **Martin Luther King junior medical achievement award** for his contributions to the study of sickle cell anaemia.

The **1972 Lenin Prizes for Science and Technology** were awarded as follows: to **Dr Zhores I. Alferov**, of the "A. F. Ioffe" Institute of Physics and Technology of the Soviet Academy of Sciences, and to his team **Vyacheslav M. Andreev**, **Dmitrii Z. Garbuzov**, **Vladimir I. Korol'kov**, **Vasilii I. Shveikin** and **Dmitrii N. Tret'yakov**, for fundamental research

on heterotransitions in semiconductors and the development of new devices based on this research; to **Dr Vadim V. Afrosimov**, **Dr Vladimir M. Dukel'skii** and **Dr Nikolai V. Fedorenko** of the "A. F. Ioffe" Institute of Physics and Technology of the Soviet Academy of Sciences, and to **Dr Oleg B. Firsov** and **Vsevolod A. Belyaev** of the "I. V. Kurchatov" Institute of Atomic Physics, for a cycle of works on "Elementary Processes and Inelastic Scatter in Atomic Collisions", published 1951–1970; to **Academician Aleksei Z. Petrov** of the Institute of Theoretical Physics of the Ukrainian Academy of Sciences, for a cycle of works on "Invariant-group Methods in the Theory of Gravitation"; to **Academician Ivan M. Vinogradov**, director of the "V. I. Steklov" Institute of Mathematics of the Soviet Academy of Sciences, for his monograph "Method of Trigonometric Sums in the Theory of Numbers", published in 1971; to **Academician Ivan L. Knunyants** of the Institute of Elemental Organic Compounds of the Soviet Academy of Sciences, for research on fluoro-organic compounds of the aliphatic series; to **Academician Vladimir I. Smirnov**, faculty head at the "M. V. Lomonosov" Moscow State University, **Academician Georgii S. Dzotsenidze** of the Geological Institute of the Georgian Academy of Sciences, and **Dr Vasilii N. Kotlyar**, faculty head at the Moscow "S. Ordzhonikidze" Institute of Geology and Prospecting, for various works on the ore-content of volcanic formations; to **Academician Petr K. Anokhin**, faculty head at the First Moscow "I. M. Sechenov" Institute, for his monograph "The Biology and Neurophysiology of the Conditioned Reflex", published in 1968; to **Academician Aleksandr I. Baraev** of the Lenin All-Union Academy of Agricultural Sciences, and his team, **Aleksandr A. Zaitseva** (All-Union Scientific Research Institute of Cereal Crops), **Georgii G. Berestovskii** (Pavlodar Soil Conservation and Erosion Research Station), **Aleksandr A. Plishkin** (All-Union Scientific Research Institute of Mechanization of Agriculture) and **Ivan I. Khoroshilov** (Soviet Ministry of Agriculture), for work on protection against wind erosion of soils in the North Caucasus and West Siberia; to **Akop A. Sarkisov** and his team, **Evgenii I. Ozerskii** (Central Asian Irrigation Control), **Abdulkarim K. Kasimov** (Tadzhiktselinstroi), **Tukhtamyshev Baimirov** (Golodnostepstroii), **Dmitrii K. Tersitskii** (director of the "Sredazgiprovodkhopok" Institute), **Emmanuil M. Ben'yaminovich** (of the same Institute), for research and implementation of irrigation methods for the virgin steppe; to **Dr Boris S. Balakshin**, faculty head at the Moscow Institute of Machine Tools and Instruments, and his team, **Boris M. Bazrov**, **Sergei P.**

Protopopov, Evgenii I. Lutskov, Yurii M. Solomentsev and Vladimir A. Timiryazev, for research into new methods for increasing precision in the machine-tool industry using adaptive control systems; to Corresponding-Academician Stanislav V. Emel'yanov, deputy director of the Institute of Problems of Control (Automation and Telemechanics) and Vadim I. Utkin of the same Institute, for a cycle of works on variable-structure systems.

Errata

Fig. 3 in the article "Smoking, Platelets and Thrombosis" by Rosemary I. Hawkins (*Nature*, 236, 450; 1972) showed human platelet electrophoretic mobility instead of the comparative thromboelastograms of a non-smoking subject and a heavy smoker (more than twenty cigarettes per day). The correct Fig. 3 is given here, showing the hypercoagulable thromboelastographic pattern in the smoking subject.

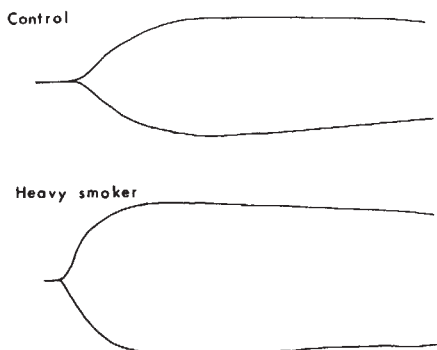


Fig. 3 Thromboelastograph recordings of a non-smoking subject (upper tracing) compared with a heavy smoker (lower tracing).

In the article "Australia Antigen as a Marker of Propagation of the Serum Hepatitis Virus in Liver Cultures" by

A. J. Zuckerman, Pamela M. Baines and June D. Almeida (*Nature*, 236, 78; 1972), the second sentence of paragraph 4 should read "The tissue was . . . cut into fragments of approximately 2 mm³".

In the review "Birds" (*Nature*, 236, 412; 1972), the price of *The Status of Birds in Britain and Ireland* was mistakenly given as £3.75. The correct price is £3.00.

Reports and Publications

not included in the Monthly Books Supplement

Great Britain and Ireland

- Heriot-Watt University. Annual Report 1970/1971. Pp. 104. (Edinburgh: Heriot-Watt University, 1972.) [222]
- National Institute of Industrial Psychology. Annual Report and Statement of Accounts for the year ended 30 September 1971. Pp. 34. (London: National Institute of Industrial Psychology, 1972.) [222]
- The Polluters: Industry or Government? By Neil H. Jacoby and F. G. Pennance. (Occasional Paper No. 36.) Pp. 55. (London: Institute of Economic Affairs, 1972.) 40p. [222]
- PEP Broadsheet 534: The Costs and Benefits of Family Planning: a Study of the Financial Implications for the Public Authorities Responsible for the Health and Welfare Services. By W. A. Laing. Pp. iii+23. (London: PEP, 1972.) 60p. [222]
- University of Oxford. Museum of the History of Science. Catalogue 1: Chemical Apparatus. By C. R. Hill. Pp. vi+77. (Oxford: Museum of the History of Science, The University, 1971.) [222]
- University of Oxford. Annual Reports 1970-1971. Pp. 27. (Supplement No. 2 to the *University Gazette*, vol. cii, February 1972.) (Oxford: The University, 1972.) 50p. [222]
- The Relativity of Thought. By Harold W. Briggs. Pp. 40. (Tilghurst, Reading: H. W. Briggs, Reading Organization for Research into Elementary Time Travel, 188 Dee Road, 1971.) 20p. [222]
- Chemosphere: Chemistry, Physics and Biology as Focused on Environmental Problems, Vol. 1, No. 1, January 1972. Published bi-monthly. Pp. 1-56. Subscription, post paid (air mail charges extra) £14; \$35 per annum, for multiple-reader institutions. (Oxford and New York: Pergamon Press, 1972.) [232]
- Forestry Commission. Research and Development Paper No. 83: Trees are for People: a Transatlantic Study of Methods and Techniques for the Educational Aspects of Wildlife Conservation and Outdoor Recreation in Forests. By W. Grant. Pp. iii+25. (London: Forestry Commission, 1971.) [232]
- The Physics Exhibition Handbook 1972. Pp. 166. (London: The Institute of Physics, 1972.) [232]
- Forestry Commission. Fifty-first Annual Report and Accounts, 1970/1971. Pp. 92+7 plates. 75p. Forestry Commission Booklet No. 29: Wildlife Conservation in Woodlands. By R. C. Steele. Pp. 67. 40p net. Forestry Commission Bulletin No. 45: Windblow of Scottish Forests in January 1968. Edited by B. W. Holtam. Pp. 51. 45p net. (London: HMSO, 1971 and 1972.) [232]

Other Countries

- World Health Organization. Vector Control in International Health. Pp. vi+144. (Geneva: WHO; London: HMSO, 1972.) 32 Sw. francs; £3.20; \$8. [222]
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